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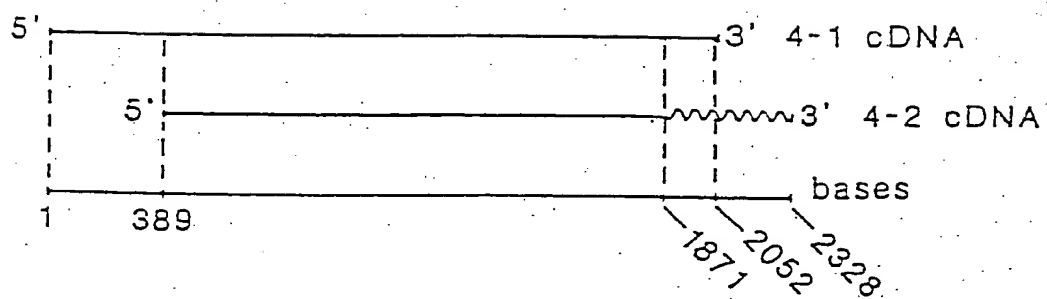


FIG. 1

10 30 50
 66C ACC 66G 6C6 CC6 CC6 CC6 CT6 CT6 CTA CT6 CC6 CT6 CT6 CT6 CTC CTA 66G ACC 66C
 Gly Thr Gly Ala Pro Pro Pro Leu Leu Leu Leu Pro Leu Leu Leu Leu Leu Gly Thr Gly
 70 90 110
 CTC TTG CCT 6CT AGC AGC CAC ATA GAG ACC CGG GCC CAT 6CG GAG GAG 6CG CTC CTG AAG
 Leu Leu Pro Ala Ser Ser His Ile Glu Thr Arg Ala His Ala Glu Glu Arg Leu Leu Lys
 130 150 170
 ASA CTC TTC TCC 6GT TAC AAC AAG TGG TCT CGG CCA GTA 6GC AAT ATC TCA GAT 6TG 6TC
 Arg Leu Phe Ser Gly Tyr Asn Lys Trp Ser Arg Pro Val Gly Asn Ile Ser Asp Val Val
 190 210 230
 CTC GTC 6GC TTT 6GC TTG TCC ATT 6CT CAG CTC ATT 6AC 6TG 6AC GAG AAG AAC CAG ATG
 Leu Val Arg Phe Gly Leu Ser Ile Ala Glu Leu Ile Asp Val Asp Glu Lys Asn Glu Met
 250 270 290
 ATG ACA ACC AAC 6TG TGG 6TG AAG CAG GAG TGG CAC 6AC TAC AAG CTG 6GC TGG GAC CCT
 Met Thr Thr Asn Val Trp Val Lys Glu Glu Trp His Asp Tyr Lys Leu Arg Trp Asp Pro
 310 330 350
 6GT GAC TAC GAG AAT 6TC ACC TCC ATC 6GC ATC CCC TCT 6AA CTC ATC TGG AGG CCT 6AC
 Gly Asp Tyr Glu Asn Val Thr Ser Ile Arg Ile Pro Ser Glu Leu Ile Trp Arg Pro Asp
 370 390 410
 ATC GTC CTC TAC AAC AAT 6CG GAT 6GA GAC TTT 6CA 6TC ACC CAC CTG ACC AAG 6CC CAC
 Ile Val Leu Tyr Asn Asn Ala Asp Gly Asp Phe Ala Val Thr His Leu Thr Lys Ala His
 430 450 470
 CTG TTC TAT GAC 6GA AGG 6TG CAG TGG ACA CCC CCA GCC ATC TAT AAG AGC TCC TGC AGC
 Leu Phe Tyr Asp Gly Arg Val Glu Trp Thr Pro Pro Ala Ile Tyr Lys Ser Ser Cys Ser
 490 510 530
 ATC GAC GTC ACC TTC TTC CCC TTT 6AC CAG CAG AAC TGT ACC ATG AAG TTT 6GA TCC TGG
 Ile Asp Val Thr Phe Phe Pro Phe Asp Glu Glu Asn Cys Thr Met Lys Phe Gly Ser Trp
 550 570 590
 ACC TAC 6AC AAG GCC AAG ATT GAC TTA 6TG AGC ATT CAT AGC 6GT 6TG 6AC CAA CTG 6AC
 Thr Tyr Asp Lys Ala Lys Ile Asp Leu Val Ser Ile His Ser Arg Val Asp Glu Leu Asp
 610 630 650
 TTC TGG 6AA 6GT 6GG 6AG TGG GTC ATC 6TG 6AT 6CT 6TG 6GC ACC TAC AAC ACC AGG AAG
 Phe Trp Glu Ser Gly Glu Trp Val Ile Val Asp Ala Val Gly Thr Tyr Asn Thr Arg Lys
 670 690 710
 TAC 6AG TGC TGT GCC 6AG ATC TAT CCT 6AC ATC ACC TAT 6CC TTC ATC ATC 6GA 6GC CTG
 Tyr Glu Cys Cys Ala Glu Ile Tyr Pro Asp Ile Thr Tyr Ala Phe Ile Ile Arg Arg Leu
 730 750 770
 CC6 CTA TTC TAC ACC ATC AAC CTC ATC ATC CC6 TGC CTG CTC ATC TCC TGT CTC ACC 6TG
 Pro Leu Phe Tyr Thr Ile Asn Leu Ile Ile Pro Cys Leu Leu Ile Ser Cys Leu Thr Val

FIG. 2A

790 810 830
 CTG GTC TTC TAT CTG CCT TCA GAG TGT GGC GAG AAG GTC ACA CTG TGC ATC TCG GTG CTG
 Leu Val Phe Tyr Leu Pro Ser Glu Cys Gly Glu Lys Val Thr Leu Cys Ile Ser Val Leu
 850 870 890
 CTT TCT CTC ACC GTC TTC CTG CTG CTC ATC ACC GAG ATC ATC CCG TCC ACC TCG CTG GTG
 Leu Ser Leu Thr Val Phe Leu Leu Leu Ile Thr Glu Ile Ile Pro Ser Thr Ser Leu Val
 910 930 950
 ATC CCG CTC ATC GGC GAG TAC CTC CTC TTC ACC ATG ATC TTC GTC ACC CTC TCC ATC GTG
 Ile Pro Leu Ile Gly Glu Tyr Leu Leu Phe Thr Met Ile Phe Val Thr Leu Ser Ile Val
 970 990 1010
 ATC ACG GTC TTC GTG CTC AAT GTG CAC CAC CCG TCG CCA CCG ACA CAC ACG ATG CCC GCG
 Ile Thr Val Phe Val Leu Asn Val His His Arg Ser Pro Arg Thr His Thr Met Pro Ala
 1030 1050 1070
 TGG GTG CGT AGA GTC TTC CTG GAC ATC GTG CCT CCG CTC CTC TTC ATG AAG CCG CCC TCT
 Trp Val Arg Arg Val Phe Leu Asp Ile Val Pro Arg Leu Leu Phe Met Lys Arg Pro Ser
 1090 1110 1130
 GTG GTC AAA GAC AAC TGC CCG AGA CTT ATT GAG TCC ATG CAC AAG ATG GCC AAC GCG CCC
 Val Val Lys Asp Asn Cys Arg Arg Leu Ile Glu Ser Met His Lys Met Ala Asn Ala Pro
 1150 1170 1190
 CCG TTC TGG CCA GAG CCT GTG GGC GAG CCC GGC ATC TTG AGT GAC ATC TGC AAC CAA GGT
 Arg Phe Trp Pro Glu Pro Val Gly Glu Pro Gly Ile Leu Ser Asp Ile Cys Asn Gln Gly
 1210 1230 1250
 CTG TCA CCT GCC CCA ACT TTC TGC AAC CCC ACG GAC ACA GCA GTC GAG ACC CAG CCT ACG
 Leu Ser Pro Ala Pro Thr Phe Cys Asn Pro Thr Asp Thr Ala Val Glu Thr Gln Pro Thr
 1270 1290 1310
 TGC AGG TCA CCC CCC CTT GAG GTC CCT GAC TTG AAG ACA TCA GAG GTT GAG AAG GCC AGT
 Cys Arg Ser Pro Pro Leu Glu Val Pro Asp Leu Lys Thr Ser Glu Val Glu Lys Ala Ser
 1330 1350 1370
 CCC TGT CCA TCG CCT GGC TCC TGT CCT CCA CCC AAG AGC AGC AGT GGG GCT CCA ATG CTC
 Pro Cys Pro Ser Pro Gly Ser Cys Pro Pro Pro Lys Ser Ser Ser Gly Ala Pro Met Leu
 1390 1410 1430
 ATC AAA GCC AGG TCC CTG AGT GTC CAG CAT GTG CCC AGC TCC CAA GAA GCA GCA GAA GAT
 Ile Lys Ala Arg Ser Leu Ser Val Gln His Val Pro Ser Ser Gln Glu Ala Ala Glu Asp
 1450 1470 1490
 GGC ATC CCG TGC CCG TCT CCG AGT ATC CAG TAC TGT GTT TCC CAA GAT GGA GCT GCC TCC
 Gly Ile Arg Cys Arg Ser Arg Ser Ile Gln Tyr Cys Val Ser Gln Asp Gly Ala Ala Ser
 1510 1530 1550
 CTG GCT GAC AGC AAG CCC ACC AGC TCC CCG ACC TCC CTG AAG GCC CCG CCA TCC CAG CTT
 Leu Ala Asp Ser Lys Pro Thr Ser Ser Pro Thr Ser Leu Lys Ala Arg Pro Ser Gln Leu

FIG. 2B

1570
 CCC GTG TCA GAC CAG GCC TCT CCA TGC AAA TGC ACA TGC AAG GAA CCA TCT CCT GTG TCC
 Pro Val Ser Asp Gln Ala Ser Pro Cys Lys Cys Thr Cys Lys Glu Pro Ser Pro Val Ser
 1590
 CCA GTC ACT GTG CAC AAG GCG GGA GGC ACC AAA GCA CCT CCC CAA CAC CTG CCC CTG TCA
 Pro Val Thr Val Leu Lys Ala Gly Gly Thr Lys Ala Pro Pro Gln His Leu Pro Leu Ser
 1630
 CCA GCC CTG ACA CGG GCA GTA GAA GGC GTC CAG TAC ATT GCA GAC CAC CTC AAG GCA GAA
 Pro Ala Leu Thr Arg Ala Val Glu Gly Val Gln Tyr Ile Ala Asp His Leu Lys Ala Glu
 1650
 GAC ACT GAC TTC TCG GTG AAG GAG GAC TGG AAA TAC GTG GCC ATG GTC ATT GAC CGA ATC
 Asp Thr Asp Phe Ser Val Lys Glu Asp Trp Lys Tyr Val Ala Met Val Ile Asp Arg Ile
 1670
 TTC CTC TGG ATG TTC ATC ATT GTC TGC CTT CTG GGC ACT GTG GGA CTC TTC CTG CCT CCC
 Phe Leu Trp Met Phe Ile Ile Val Cys Leu Leu Gly Thr Val Gly Leu Phe Leu Pro Pro
 1690
 TGG CTG GCT GCT TGC TGA TGGCTTCGACAGTGTCTCAAGGCTCACGTCTCTGCTGACTTTGTTTCCAG
 Trp Leu Ala Ala Cys
 1710
 TTTCTTCTCCGACAAAGTTGGCCTCCCTTCATTATTTCTGTTATTTTGGGCTTCGTGTTATTAATATCCTTCCCTGCC
 1730
 TCTGTGGCGCATTGTAAGTTTTAAAAATTAATAGACCAAAGCC...3'
 4-2 cDNA: 3' end
 1867
 CCC TGG CTG GCT GGT ATG ATC TAG
 Pro Trp Leu Ala Gly Met Ile
 1884
 GGACGTGGTGGTGGCCAGCTCCACATCTCTGTAAGGCCATAC
 1912
 GACTGCTCAGTCACCCACATCTTCCAAACCGGCTGACCATGAGACACCCTAGGAGAGAGATGATGCTTCTTGGGAGATG
 1937
 GAAGTTGGCCCTGGTTCTAGTCAGACTATGGGCGTGGTTGGAGAGAAATGAGGGCTGATACAGTTGCAAGCCGAGTCCC
 2016
 CATTAAAGTTTCTCCAGAGCAAGTGGCAGTACTCCCTGACTTACAGACAGCACACCCATCTGTGTACAGAGAATGA
 2042
 TCCCAGATTGATCTCAGTTGTCTTTGAGGCCATGAAAAATTCATCCACCTTGAGGAACCAGAGGCTCTCATGCTGTGG
 2070
 GATCAATAAGACCAAGGAATCTCCCACTGTGACTCTGCTGGCCACACCCTCTCCCTCCCCAAGAAGTGGTCCCTCATCC
 2095
 CCAATTC...3'
 2121
 2149
 2174
 2200
 2228
 2253
 2279
 2307

FIG. 2C

[illegible]

FIG. 3A

180 Asp Phe Trp Glu Ser Gly Trp Val Ile Val Asp Ala Val Gly Thr Tyr Asn Thr Arg Lys Tyr Glu Cys Glu Ile Tyr Pro
 GAC TTC TGG GAA AGT GGC GAG GAG GTC ATC ATC GAT GCT GGT GGG ACC TAC AAC ACC AGG AAG TAC GAG TGC TGT GCC GAG ATC TAT CCT
 540
 210 Asp Ile Thr Tyr Ala Phe Ile Ile Arg Arg Leu Pro Leu Phe Tyr Thr ACC ATC AAC CTC ATC ATC CCG CCG TGC CTC Cys Leu Ile Ser Cys Leu Thr
 GAC ATC ACC TAT GCC TTC ATC ATC GCA CGC CGC CTG CCG CTA TTC TAC ACC ATC AAC CTC ATC ATC CCG TGC TGC CTC ACC TCC TGT CTC ACC
 630
 240 Val Leu Val Phe Tyr Ile Pro Ser Glu Cys Gly Gly Val Lys Val Thr Leu Cys Ile Ser Val Leu Leu Ser Leu Thr Val Phe Leu Leu Leu
 GTG CTG ATC TTC TAT CTG CCT TCA GAG TGT GGC GAG AAG GTC ACA CTG TGC ATC TCG GTG CTG CTT TCT CTC ACC TTC GTC CTC CTC CTC
 720
 270 Ile Thr Glu Ile Ile Pro Thr Ser Leu Val Ile Pro Leu Ile Gly Glu Tyr Leu Leu Phe Thr Met Ile Phe Val Thr Leu Ser Ile
 ATC ACC GAG ATC ATC CCG TCC ACC TCG CTG GTC ATC CCG CTC ATC GGC GAG TAC CTC CTC ACC ATG ATC TTC TGC ACC CTC TCC ATC
 810
 300 Val Ile Thr Val Phe Val Leu Asn Val His His Arg Ser Pro Arg Thr His Thr Met Pro Ala Trp Val Arg Arg Val Phe Leu Asp Ile
 GTC ATC ACG GTC TTC GTG CAC CAC CAC CGC TCG CCA CGC ACA CAC CAC AGG ATG CCC GGC TGG GTG CGT AGA GTC GTC TTC CTC GAC ATC
 900
 330 Val Pro Arg Leu Leu Phe Met Lys Arg Pro Ser Val Val Lys Asp Asn Cys Arg Arg Leu Ile Glu Ser Met His Lys Met Ala Asn Ala
 GTG CCT CGC CTC CTC ATG AAG CGC CCC TCT GTG GTC AAA GAC AAC TGC CGG AGA CTT ATT GAG TCC CTC TCA CCA CCA CCA CCA CCA CCA
 990
 360 Pro Arg Phe Trp Pro Glu Val Glu Gly Pro Gly Ile Leu Ser Asp Ile Cys Asn Gln Gly Leu Ser Pro Ala Thr Phe Cys Asn
 CCC CGC TTC TGG CCA GAG CCT GTG GGC GAG CCC GGC ATC TTG AGT GAC ATC TGC AAC CAA GGT CTG TCA CCT GCC CCA CCA CCA CCA CCA CCA
 1080
 390 Pro Thr Asp Thr Ala Val Glu Thr Gln Pro Thr Cys Arg Ser Pro Pro Glu Thr Ser Glu Val Val Glu Lys Ala
 CCC ACG GAC ACA GCA GTC GAG ACC CAG CCT ACG TGC AGG TCA CCC CCC CCC CTT GAG GTC CCT GAC TTG AAG ACA TCA TCA TCA TCA TCA TCA TCA
 1170
 410 Val Glu Lys Ala
 GAG GAT GAG AAG GCC

FIG. 3B

[illegible]

FIG. 3C

ALPHA4 G T G A P P P L L L L P L L L L L G T G L L P A S S H
 ALPHA3 M G V V L L P P P L S M L M L V L M L L P A A S A
 ALPHA1 M E L S T V L L L L G L S S A G L V L G
 signal peptide

ALPHA4 I E T R A H A E E R L L K R L F S G Y N K W S R P V G
 ALPHA3 - - - - S E A E H R L P Q Y L F E D Y N E I I R P V A
 ALPHA1 - - - - S E H E T R L V A K L F E D Y S S V V R P V E

ALPHA4 N I S D V V L V R F G L S I A Q L I D V D E K N O M M
 ALPHA3 N V S H P V I I Q F E V S M S Q L V K V D E V N O I M
 ALPHA1 D H R E I V Q V T V G L Q L I Q L I N V D E V N O I V

ALPHA4 T T N V W V K Q E W H D Y K L R W D P G D Y E N V T S
 ALPHA3 E T N L W L K Q I W N D Y K L K W K P S D Y Q G V E F
 ALPHA1 T T N V R L K Q Q W V D Y N L K W N P D D Y G G V K K

ALPHA4 I R I P S E L I W R P D I V L Y N N A D G D F A V T H
 ALPHA3 M R V P A E K I W K P D I V L Y N N A D G D F Q V D D
 ALPHA1 I H I P S E K I W R P D V V L Y N N A D G D F A I V K

ALPHA4 L T K A H L F Y D G R V Q W T P P A I Y K S S C S I D
 ALPHA3 K T K A L L K Y T G E V T W I P P A I F K S S C K I D
 ALPHA1 F T K V L L D Y T G E H I T W T P P A I F K S Y C E I I

ALPHA4 V T F F P F D Q Q N C T M K F G S W T Y D K A K I D L
 ALPHA3 V T Y F P F D Y Q N C T M K F G S W S Y D K A K I D L
 ALPHA1 V T H F P F D E Q N C S M K L G T W T Y D G S V V A I

ALPHA4 V S I H S R V D Q L D F W E S G E W V I V D A V G T Y
 ALPHA3 V L I G S S M N L K D D Y W E S G E W A I I K A P G Y K
 ALPHA1 N P E S D O P D L S N F M E S G E W V I K E A R G W X

ALPHA4 N T R K Y E C C - A E I Y P D I T Y A F I I R R L P L
 ALPHA3 H E I K Y N C C - E E I Y O D I T Y S L Y I R R L P L
 ALPHA1 H W V F Y S C C P T T P Y L D I T Y H F V M Q R L P L

ALPHA4 F Y T I N L I I P C L L I S C L T V L V F Y L P S E C
 ALPHA3 F Y T I N L I I P C L L I S F L T V L V F Y L P S D C
 ALPHA1 Y F I V N V I I P C L L F S F L T S L V F Y I P T D S
 -----MSR I----->

ALPHA4 G E K V T L C I S V L L S L T V F L L L I T E I I P S
 ALPHA3 G E K V T L C I S V L L S L T V F L L V I T E T I P S
 ALPHA1 G E K M T L S I S V L L S L T V F L L V I V E L I P S
 <-----MSR II----->

ALPHA4 T S L V I P L I G E Y L L F T M I F V T L S I V I T V
 ALPHA3 T S L V I P L I G E Y L L F T M I F V T L S I V I T V
 ALPHA1 T S S A V P L I G K Y M L F T M V F V I A S I I I T V
 -----MSR III----->

FIG. 4A

ALPHA4	F	V	L	N	V	H	E	R	S	P	R	T	H	T	M	P	A	W	V	R	R	V	F	L	D	I	V			
ALPHA3	F	V	L	N	V	H	Y	R	T	P	T	T	H	T	M	P	T	W	V	K	A	V	F	L	N	L	L			
ALPHA1	I	V	I	N	T	H	E	R	S	P	S	T	H	I	M	P	E	W	V	R	K	V	F	I	D	T	I			
----->																														
ALPHA4	P	R	L	L	F	-	-	-	M	K	R	P	S	V	V	K	D	N	C	R	R	L	I	E	S	M	H			
ALPHA3	P	R	V	M	F	-	-	-	M	T	R	P	T	S	G	E	G	D	T	P	K	T	-	-	-	-	-			
ALPHA1	P	N	I	M	F	F	S	T	M	K	R	P	S	R	D	K	Q	E	K	R	I	F	-	-	-	-	-			
----->																														
ALPHA4	X	M	A	N	A	P	R	F	W	P	E	P	V	G	E	P	G	I	L	S	D	I	C	N	Q	G	L			
ALPHA3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	T	F	Y	G	A	E	L	S	N	L	N	C	F	S	R
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	E	D	I	D	I	S	D	I	S	G	X	P	G		
----->																														
ALPHA4	S	P	A	P	T	F	C	N	P	T	D	T	A	V	E	T	Q	P	T	C	R	S	P	P	L	E	V			
ALPHA3	A	D	S	X	S	C	K	E	G	Y	P	C	O	D	G	T	C	C	Y	C	H	H	R	R	V	K	I			
ALPHA1	P	P	P	M	G	F	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
----->																														
ALPHA4	P	D	L	K	T	S	E	V	E	K	A	S	P	C	P	S	P	G	S	C	P	P	P	K	S	S	S			
ALPHA3	S	N	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	A	N	L	T	R	S	S	S			
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
----->																														
ALPHA4	G	A	P	M	L	I	K	A	R	S	L	S	V	Q	H	V	P	S	S	Q	E	A	A	E	D	G	I			
ALPHA3	S	E	S	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
----->																														
ALPHA4	R	C	R	S	R	S	I	Q	Y	C	V	S	Q	D	G	A	A	S	L	A	D	S	K	P	T	S	S			
ALPHA3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
----->																														
ALPHA4	P	T	S	L	K	A	R	P	S	Q	L	P	V	S	D	Q	A	S	P	C	K	C	T	C	K	E	P			
ALPHA3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
----->																														
ALPHA4	S	P	V	S	P	V	T	V	L	K	A	G	G	T	K	A	P	P	Q	H	L	P	L	S	P	A	L			
ALPHA3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	A	V	L	S	L	S	A	L	S	P	E	I		
ALPHA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	P	L	I	X	H	P	E	V			
<-----																														
ALPHA4	T	R	A	V	E	C	V	Q	Y	I	A	D	H	L	K	A	E	D	T	D	F	S	V	K	E	D	W			
ALPHA3	K	E	A	I	Q	S	V	X	Y	I	A	E	N	M	K	A	Q	N	V	A	K	E	I	O	D	D	W			
ALPHA1	K	S	A	I	E	C	V	K	Y	I	A	E	T	M	K	S	D	Q	E	S	N	N	A	A	E	E	W			
---phipathic helix----->																														
ALPHA4	K	Y	V	A	M	V	I	D	R	I	F	L	W	M	F	I	I	V	C	L	L	G	T	V	G	L	F			
ALPHA3	K	Y	V	A	M	V	I	D	R	I	F	L	W	V	F	I	L	V	C	I	L	G	T	A	G	L	F			
ALPHA1	K	Y	V	A	M	V	M	D	H	I	L	L	G	V	F	M	L	V	C	L	I	G	T	L	A	V	F			
<-----MSR IV----->																														
ALPHA4	L	P	P	W	L	A	G	M	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA3	L	Q	P	L	M	A	-	R	D	D	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
ALPHA1	A	G	R	L	I	E	L	H	Q	Q	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
-->																														

FIG. 4B

FIG. 5A

Clone 4-1;
Antisense

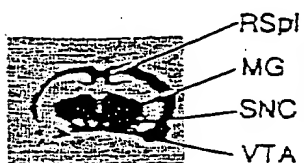
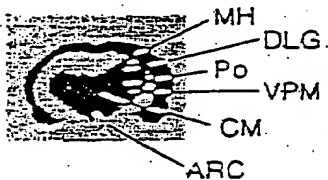
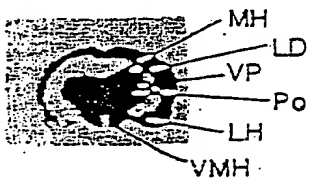
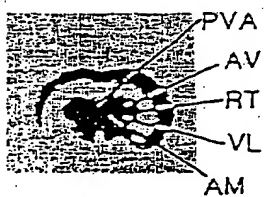


FIG. 5B

Clone 4-1;
Sense

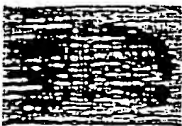
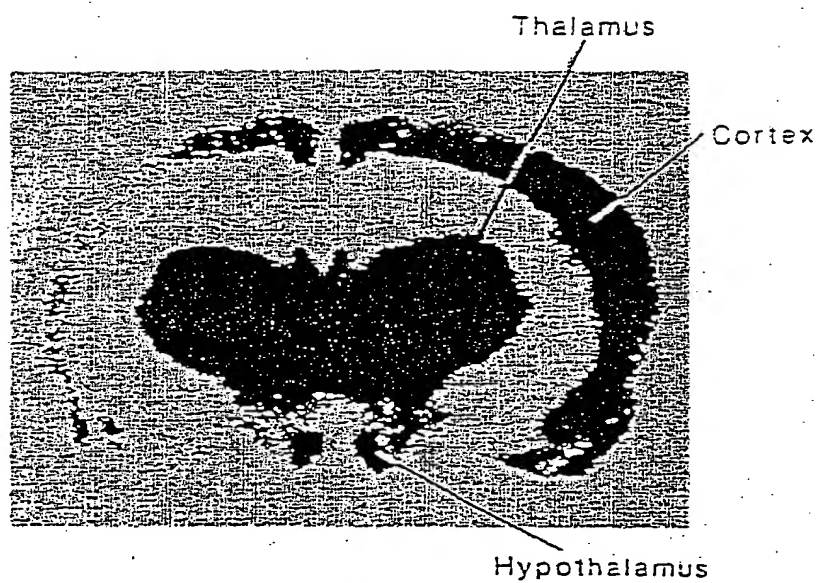
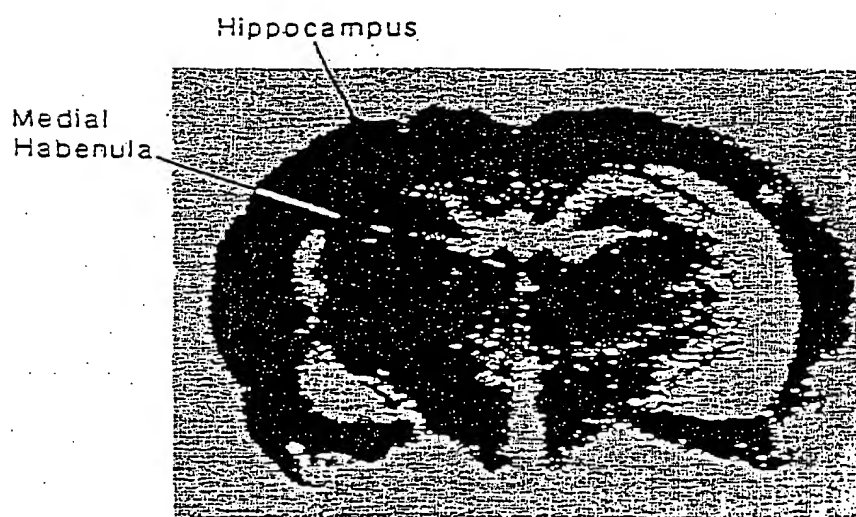


FIG. 6A



PROBE: Alpha 4

FIG. 6B



PROBE: Alpha 3

FIG. 7A

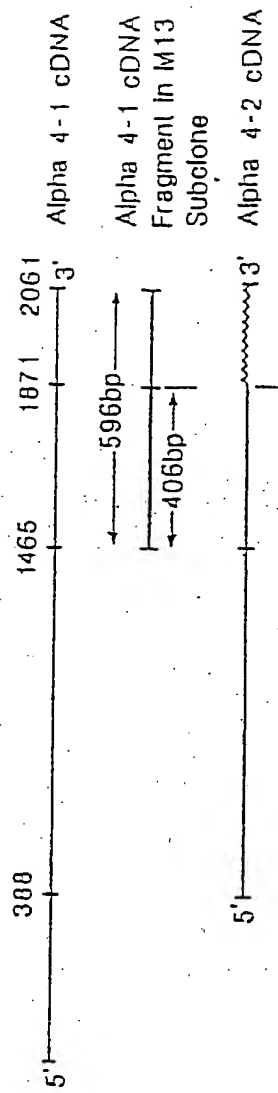


FIG. 7B

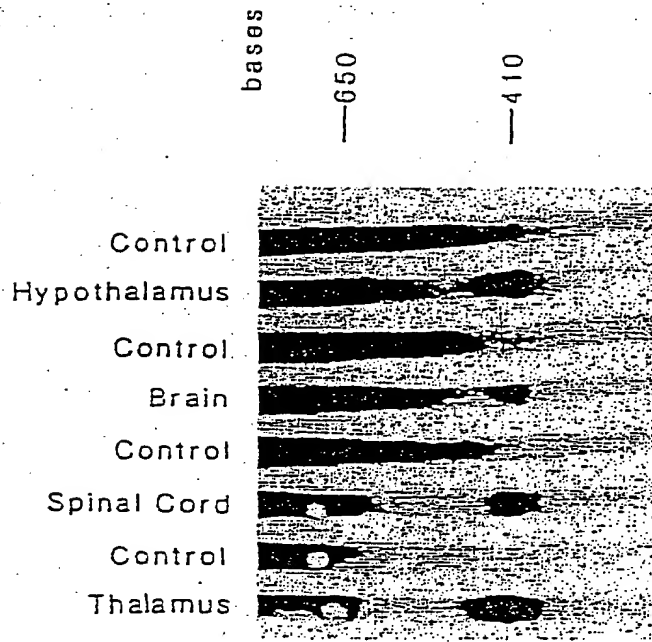
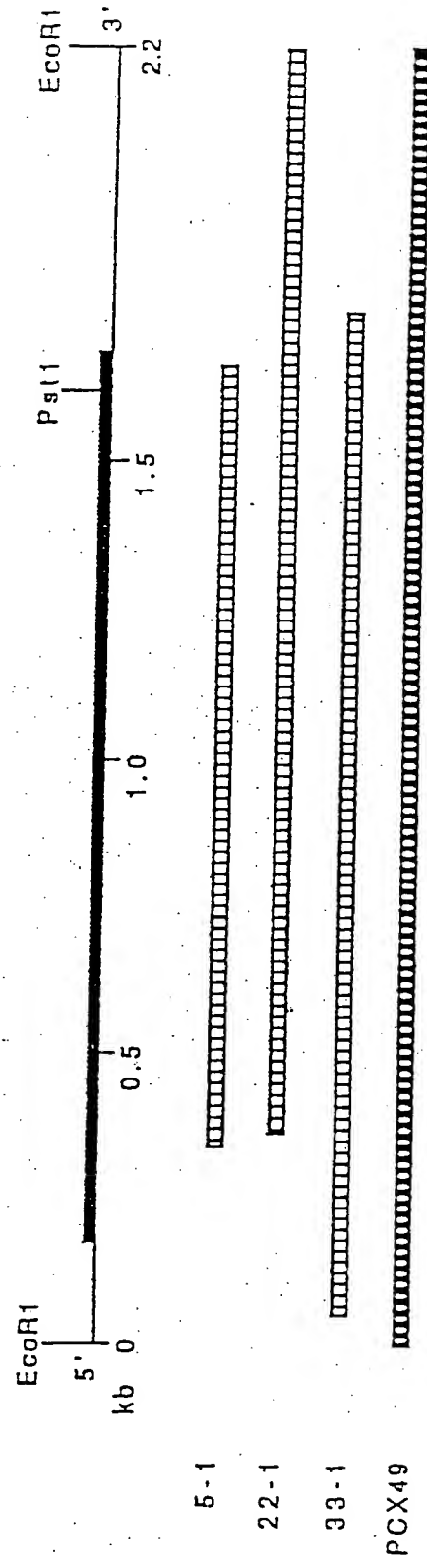


FIG. 8



-179
 5' GGGGACACACACCGGGACCGGGAGAGAGCGGGAGACCTCCCTCGTTGCAGGAACCTGCCCGTTTCAGTGAGCACCTTTAGACC
 -100
 TGGAGGCCGCGAGCCCGGAGAGCGGGGCTGCGGGGCTTCAGGACCGGAGCGGCTCGACCCGCGAGCCCTAGTATCCGAGAGGCTGCGCGGCT
 -1
 ATG CTG GCT TGC ATG GCC GGG CAC TCC AAC TCA ATG GCG CTG TTC AGC CTT CTT TGG CTG TGC TCA GGG
 Met Leu Ala Cys Met Ala Gly His Ser Asn Ser Met Ala Leu Phe Ser Phe Ser Leu Leu Trp Leu Cys Ser Gly
 1
 GTT TTG GGA ACT GAC ACA GAG GAG CCG CTA GTG GAG CAT CTC TTA GAT CCC TCC CGC TAT AAC AAG CTG ATT CGT
 Val Leu Gly Thr Asp Thr Glu Glu Arg Leu Leu His Leu Leu Asp Pro Ser Arg Tyr Asn Lys Leu Ile Arg
 26
 CCA GCT ACT AAC GGC TCT GAG CTG GAG CTG ACT GTA CAG CTC ATG GTA TCA TTG GCT CAG CTC ATT AGT GTG CAC GAG
 Pro Ala Thr Asn Gly Ser Glu Leu Val Thr Val Gln Leu Met Val Ser Leu Ala Gln Leu Ile Ser Val His Glu
 51
 CCG GAG CAG ATC ATG ACC ACC AAT GTC TGG CTG ACC CAG GAG TGG GAA GAT TAC CGC CTC ACA TGG AAG CCT GAG
 Arg Glu Gln Ile Met Thr Thr Asn Val Trp Leu Thr Gln Glu Trp Glu Asp Tyr Arg Leu Thr Trp Lys Pro Glu
 76
 GAC TTC GAC AAT ATG AAG AAA GTC CCG CTC CCT TCC AAA CAC ATC TGG CTC CCA GAT GTG GTT CTA TAC AAC AAT
 Asp Phe Asp Asn Met Lys Lys Val Arg Leu Pro Ser Lys His Ile Trp Leu Pro Asp Val Val Leu Tyr Asn Asn
 101
 GCT GAC GGC ATG TAC GAA GTC TCC TTC TAT TCC AAT GCT GTG GTC TCC TAT GAT GGC AGC ATC TTT TGG CTA CCA
 Ala Asp Gly Met Tyr Glu Val Ser Phe Tyr Ser Asn Ala Val Val Ser Tyr Asp Gly Ser Ile Phe Trp Leu Pro
 126
 CCT GCC ATC TAC AAG AGT GCA TGC AAG ATT GAG GTG AAG CAC TTC CCA TTT GAC CAG AAT TGC ACC ATG AAG
 Pro Ala Ile Tyr Lys Ser Ala Cys Lys Ile Glu Val Lys His Phe Pro Phe Asp Gln Gln Asn Cys Thr Met Lys
 151

FIG. 9A

540
 TTT CGC TCA TGG ACC TAC GAC CGT ACT GAG ATT GAC CTG GTG CTC AAA AGT GAT GTG GCC AGT CTG GAT GAC TTC
 Phe Arg Ser Trp Thr Thr Tyr Asp Arg Thr Glu Ile Asp Leu Val Leu Lys Ser Asp Val Ala Ser Leu Asp Asp Phe
 176

690
 ACA CCC AGC GGG GAG TGG GAC ATC ATC GCA CTG CCA GGC CGA CGC AAC GAG AAC CCA GAC GAC TCC ACC TAT GTG
 Thr Pro Ser Gly Glu Thr Asp Ile Ile Ala Leu Pro Gly Arg Arg Asn Glu Asn Pro Asp Asp Ser Thr Tyr Val
 201

720
 GAC ATC ACC TAT GAC TTC ATC ATT CGT CGC AAA CCA CTC TTC TAC ACT ATC AAC CTC ATC ATC CCC TGC GTA CTC
 Asp Ile Thr Tyr Asp Phe Ile Ile Arg Arg Lys Pro Leu Phe Tyr Thr Ile Asn Leu Ile Ile Pro Cys Val Leu
 226

780
 ATC ACC TCG CTG GCC ATC CTG GTC TTC TAC TAC GAC TGT GGT GAA AAG ATG ACA CTT TGT ATT TCT GTG
 Ile Thr Ser Leu Ala Ile Leu Val Phe Tyr Thr Ser Asp TCA GAC Cys Gly Glu Lys Met Thr Leu Cys Ile Ser Val
 251

840
 CTG CTA GCA CTC ACG GTG TTC CTG CTC ATC TCC AAG ATT GTG CCT CCC ACC TCC CTC GAT GTA CCG CTG GTG
 Leu Leu Ala Leu Thr Thr Val Phe Leu Leu Ile Ser Lys Ile Val Pro Thr Ser Leu Asp Val Pro Leu Val
 276

930
 GGC AAG TAC CTC ATG TTT ACC ATG GTG CTA GTC ACC TTC TCC ATC GTC ACC AGC GTG TGT GTG CTC AAT GTG CAC
 Gly Lys Tyr Leu Met Phe Thr Met Val Leu Val Thr Phe Ser Ile Val Thr Ser Val Cys Val Leu Asn Val His
 301

990
 CAC CGC TCG CCT ACC ACG CAC ACC ATG GCC CCC TGG GTC AAG GTG GTC TTC CTG GAG AAG AAG CTC CCC ACC CTG CTC
 His Arg Ser Pro Thr Thr Thr His Thr Met Ala Pro Trp Val Lys Val Val Phe Leu Glu Lys Leu Pro Thr Leu Leu
 326

1080
 TTC CTG CAG CAG CCA CGC CAC CGC TGT GCA CGT CAG CGT CTG CGC TTG AGG AGG CGC CAG CGA GAG CGT GAG GGC
 Phe Leu Gln Gln Pro Arg Arg His Arg Cys Ala Arg Gln Arg Leu Arg Arg Arg Arg Gln Arg Glu Arg Glu Gly
 351

570
 600
 660
 750
 810
 870
 900
 960
 1020
 1050
 1110

FIG. 9B

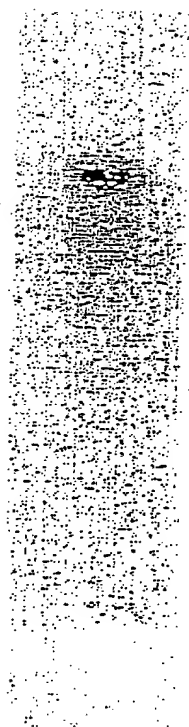
1140 GAG GCG GTT TTC TTC CGT GAA GGT CCT GCG GCT GAC CCA TGT ACC TGC TTT GTC AAC CCT GCA TCA GTG CAG GGC 1200
 Glu Ala Val Phe Phe Arg Glu Gly Pro Ala Ala Asp Pro Cys Thr Cys Phe Val Asn Pro Ala Ser Val Gln Gly
 376
 1230 TTG GCT GGG GCT TTC CGA GCT GAG CCC ACT GCA GCC GGC CCG GGG CGC TCT GTG GGG CCA TGC AGC TGT GGC CTC
 Leu Ala Gly Ala Phe Arg Ala Glu Pro Thr Ala Ala Gly Pro Gly Arg Ser Val Gly Pro Cys Ser Cys Gly Leu
 401
 1290 CCG GAA GCA GTG GAT GGC GTA CGC TTC ATT GCG GAC CAC ATG CGA AGT GAG GAT GAT GAC CAG AGT GTG AGG GAG 1350
 Arg Glu Ala Val Asp Gly Val Arg Phe Ile Ala Asp His Met Arg Ser Glu Asp Asp Arg Gln Ser Val Arg Glu
 426
 1380 GAC TGG AAA TAC GTT GCC ATG GTG ATC GAC CGC CTG TTC CTG TGG ATC TTT GTC TTT GTC TGT GTG TTT GGG ACC
 Asp Trp Lys Tyr Val Ala Met Val Ile Asp Arg Leu Phe Leu Trp Ile Phe Val Phe Val Cys Val Phe Gly Thr
 451
 1440 GTC GGC ATG TTC CTG CAG CCT CTC TTC CAG AAC TAC ACT GCC ACT ACC TTC CTC CAC CCT GAC CAC TCA GCT CCC 1500
 Val Gly Met Phe Leu Gln Pro Leu Phe Gln Asn Tyr Thr Ala Thr Thr Phe Leu His Pro Asp His Ser Ala Pro
 476
 1596 AGC TCC AAG TGAGGTCATTCATTGTCAGCTCCTCACCCCGTGACCCCTGGGTTTCAGTACTGGGTGCAAGATGGATCTCTCCCCACTCCACTGA
 Ser Ser Lys
 501
 1695 AGCCTGCTTCACACCCTCCGTTACACATAGTCCCTCCAGCCTGGAGGCTGGACCCGCTGCGCTTGTGGTCGAGCCCTTCTCCTTTCCCTCTGAGCTCGTTCA
 1794
 GGCAGGAGTGCCCAATGGTGGGGCCACGGCTGGTAAGTAGAGGCCAGAGATCACAGAGGCCACCTACCCCGGATGAGGTGCTGGAGAAAGGCCCAAGAAAG
 1893
 AGACAGAGTTATCTGTGACCTCCAAGTCATCGGAGAGGAGGAGGTAGGATAAGGGGCTCAGACTCTGGCAGAGTGGGGCTAGTACTTGGCGCCCACTCA
 1992
 CTTAAGTGAGCGACACTGGTCTGGGAGGACTCGAAGTGTGGGGAGCTCTCTGGGAGCTCGGTCTCCACCCTGTACCTCAGAGGGGCTCCAGACCCCGG
 2017
 GCTTCAGGTTCCCTTCTGCCAGTGC.....3'

FIG. 9C

[illegible]

FIG. 10

FIG. 11A



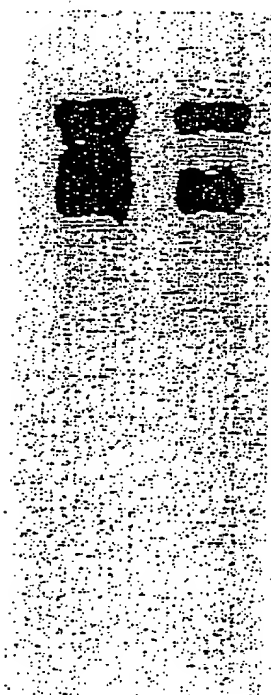
— 28S

— 18S

FIG. 11B

1

2



28S —

18S —

FIG. 12A

ANTISENSE

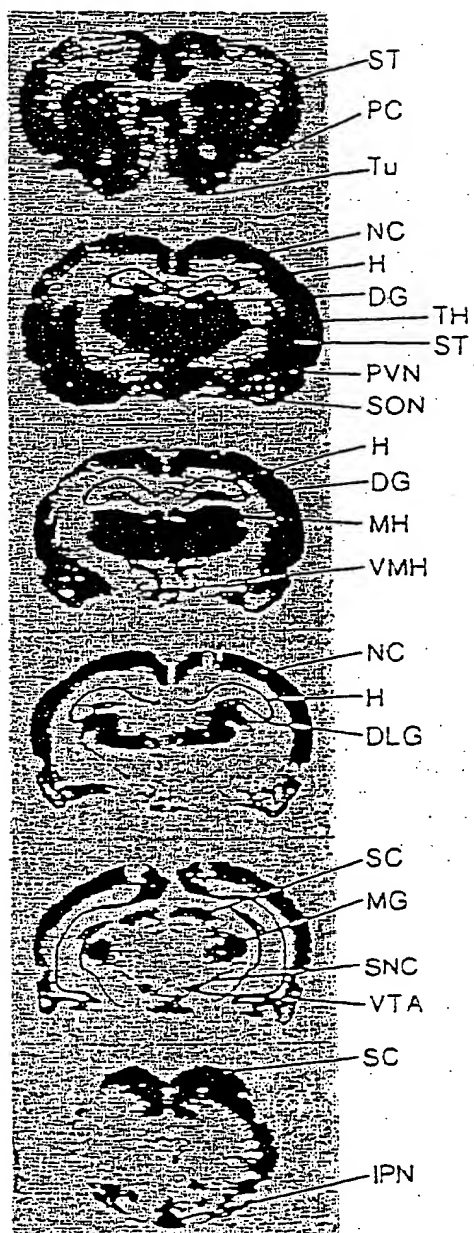
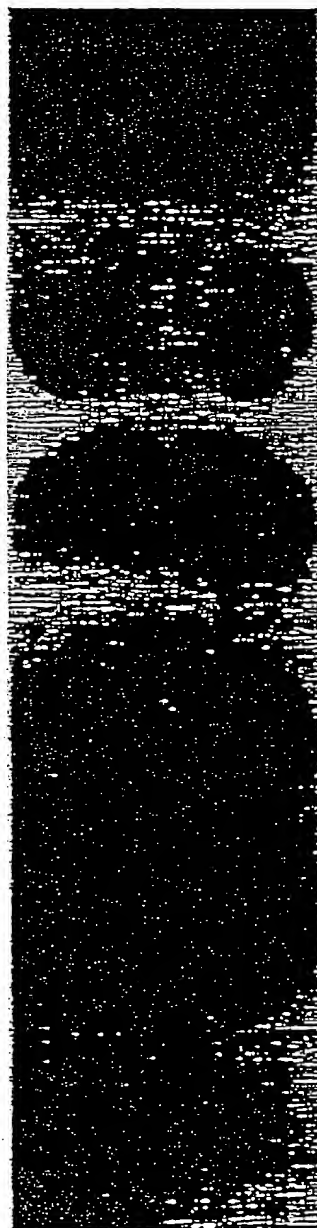


FIG. 12B

SENSE



[illegible]

FIG. 13

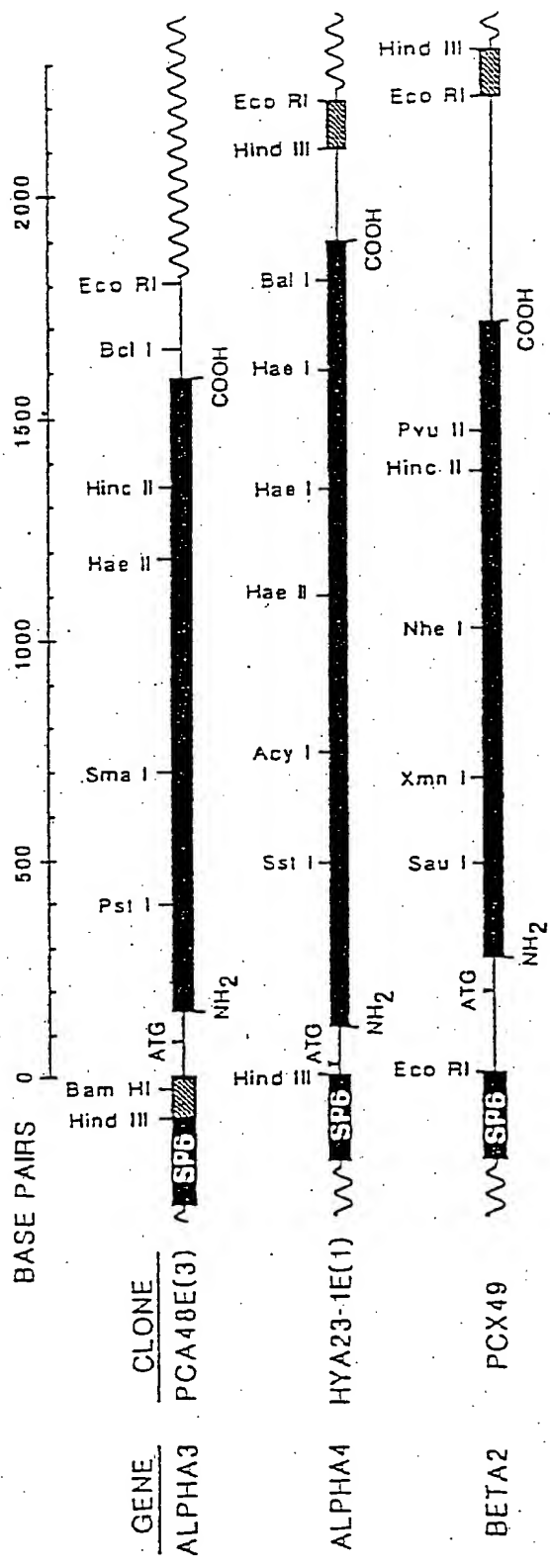


FIG. 14

FIG. 15A

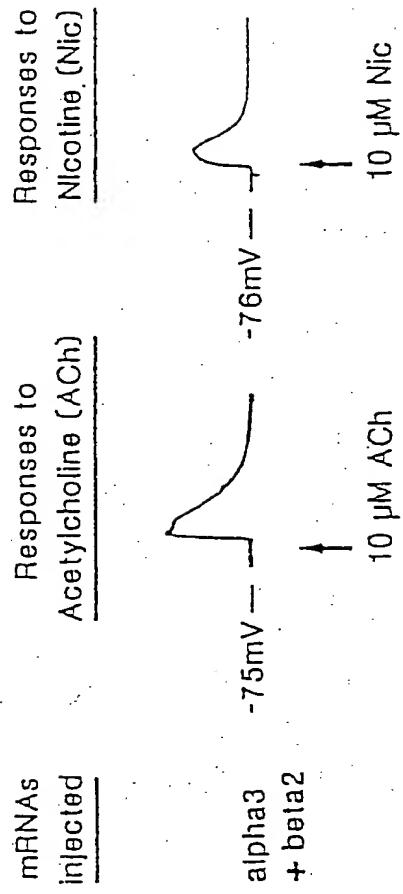


FIG. 15B

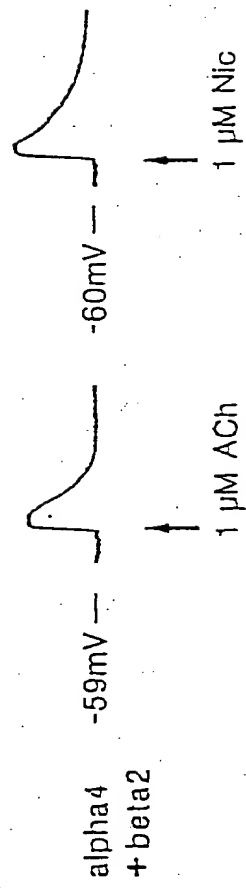


FIG. 15C

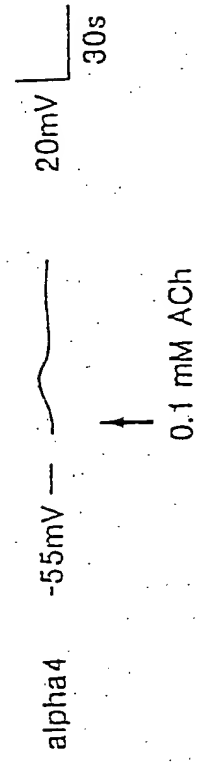


FIG. 16A

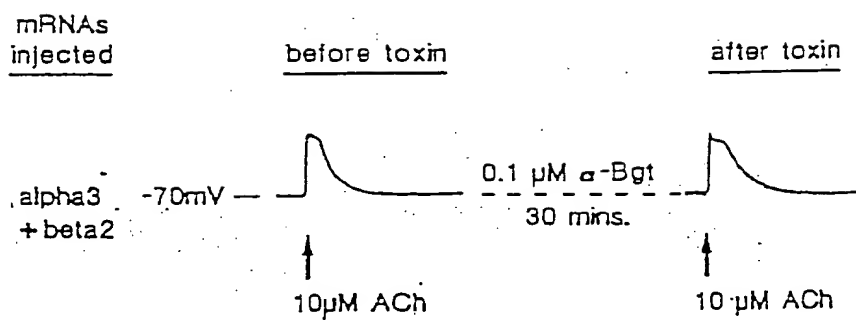


FIG. 16B

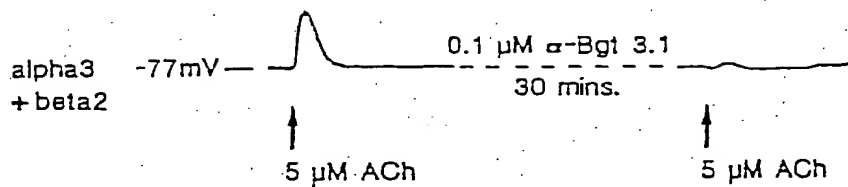


FIG. 16C

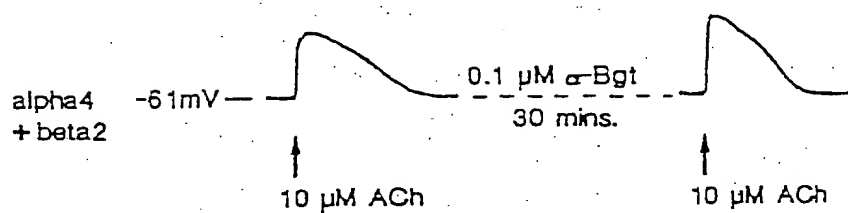
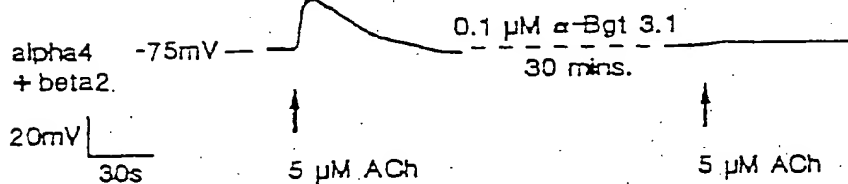


FIG. 16D



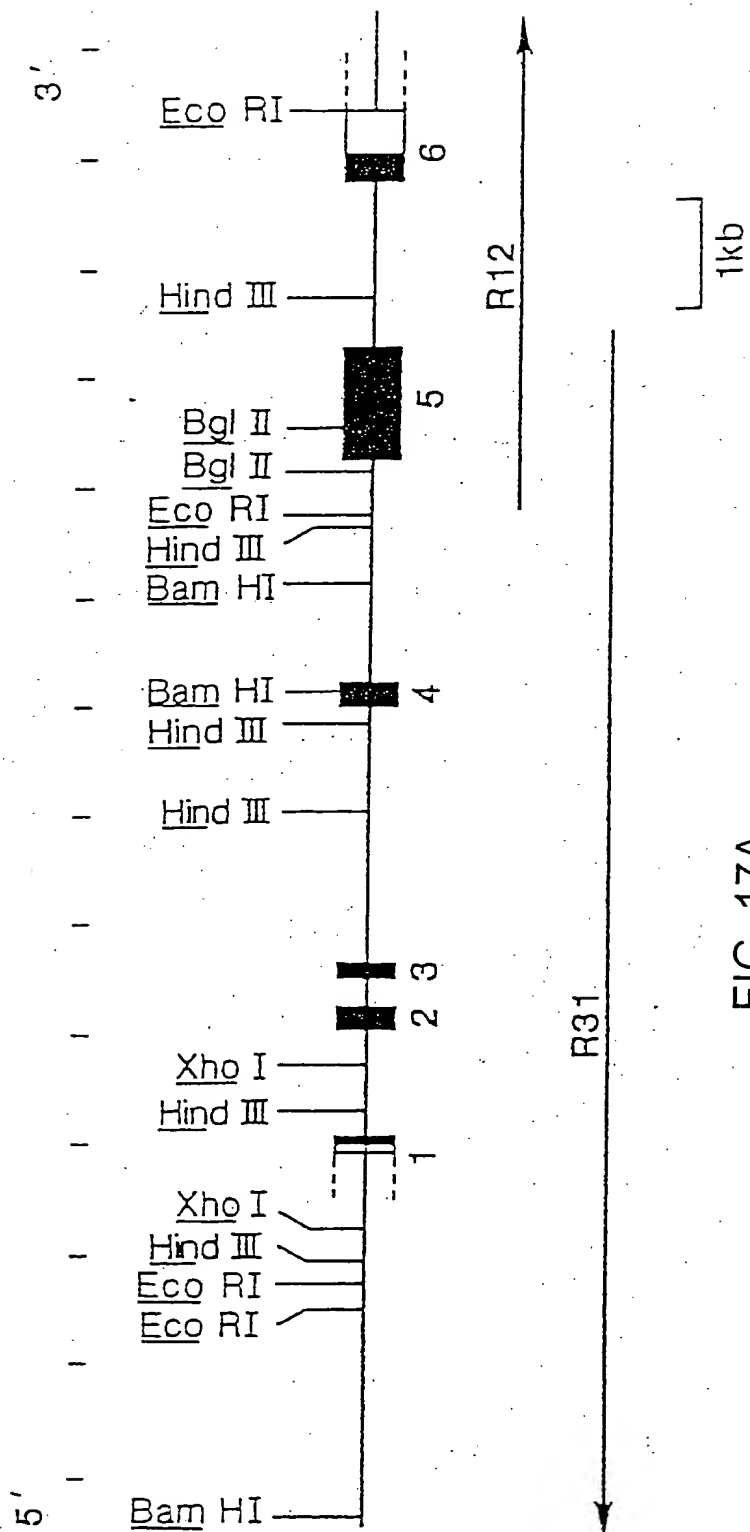


FIG. 17A

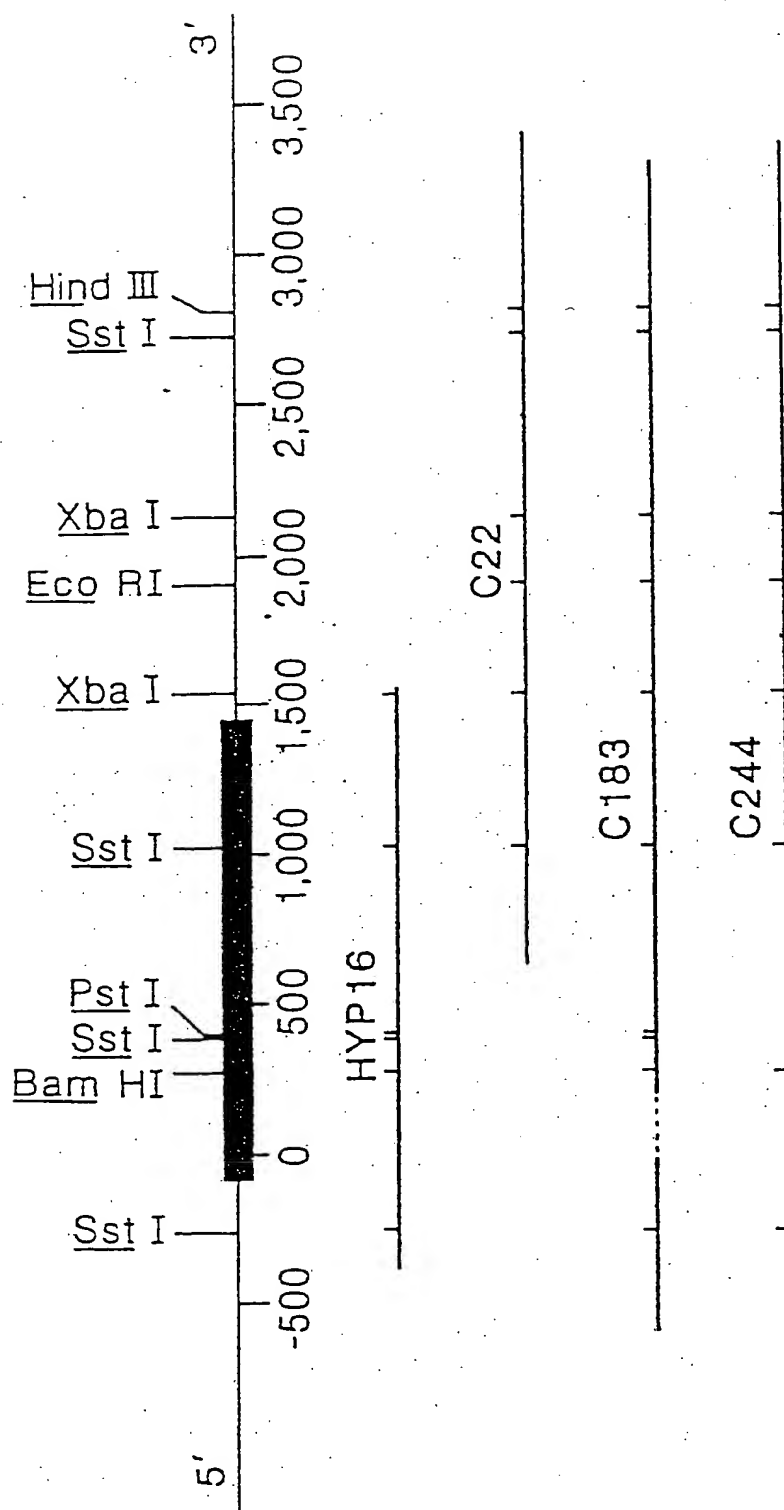


FIG. 17B

5'ACTGAGCATTG

-350 TGTGAACTCGGATCACCTATCTCCAGGAAGCTAGCCTGAATCCCTCATCTCCCAACAGTGGCTCCCAAAACCTTGCAGGTTCTGTGCTGGGCAACCATGAGCTGAAGCACTGAGCTCTG

-270

-240 TTCTGCACCTGTCTACTGCTTCGGAGGGAGCCCTCGTCAGCCACCCCAAGGTCTCCAGCCGGTTGGTTCTCTGCATCCCTTGAGGGGCCCTGTCTTCTATGACAAATTGCAGAGAGACAGT

-150

-120 GCCTCAAGAAGCCAGCTCTTGGTAGTCCAAAGGGAAACCAAGGACCCCTCTGAAGCC ATG ACC CTT ICC CAT TCT GCT CTC CAG TTC TGG ACA CAT CTT TAT CTC

-90 Met Thr Leu Ser His Ser Ala Leu Gln Phe Trp Thr His Leu Tyr Leu

-60

-30 TGG TGT CTC CTT CTG GTG CCA GCA G gtagt.....tatccacag TG TTG ACC CAG CAA GGC TCA CAC ACC CAT GCT GAG GAC CGC CTG TTC

-10 Trp Cys Leu Leu Val Pro Ala V al Leu Thr Gln Gln Gly Ser His Thr His Ala Glu Asp Arg Leu Phe

60 AAA CAC CTG TTT GGA GGC TAC AAT CGC TGG GCA CGG CCA GTG CCC AAC ACT TCT GAT GTG GTG ATC GTG CGC TTT GGA TTA ICC ATT GCT

120 Lys His Leu Phe Gly Gly Tyr Asn Arg Trp Ala Arg Pro Val Pro Asn Thr Ser Asp Val Ile Val Arg Phe Gly Leu Ser Ile Ala

150 CAG CTC ATA GAT GTG gtgggc.....gctacaacag GAT GAG AAG AAT CAA ATG ATG ACC ACC AAT GTG TGG CTA AAG CAG gtaaac.....

180 Gln Leu Ile Asp Val Asp Glu Lys Asn Gln Met Thr Thr Thr Asn Val Trp Leu Lys Gln

210ccctaagcag GAA TGG AAT GAC TAC AAG CTG CGC TGG GAC CCG GCT GAG TTT GGC AAT GTG ACC TCC CTG CGC GTC CCT TCA GAG ATG

240 Glu Trp Asn Asp Tyr Lys Leu Arg Trp Asp Pro Ala Glu Phe Gly Asn Val Thr Ser Leu Arg Val Pro Ser Glu Met

FIG. 18A

270 ATC TGG ATC CCA GAC ATT GTC CTC TAC AAC AA gtaaga.....ctcttccag T 300 GCA GAT GGG GAG TTT GCG GTG ACC CAC ATG ACC AAG
 ile Trp ile Pro Asp ile Val ile Leu Tyr Asn As 100 n Ala Asp Gly Glu Phe Ala Val Thr His Met Thr Lys
 90
 360 GCT CAC CTC TTC TTC ACC GGC ACT Thr Val His Trp Val Pro Pro Ala ile Tyr 390 AGC TCC TGC AGC ATC GAT GTG ACC TTC CCC TTC
 Ala His Leu Phe Phe Thr Gly 120 Thr Val Trp Val Trp Thr Tyr Asp Lys Ala 130 Lys Ser Ser Cys Ser ile Asp Val Thr Phe
 450 GAC CAG CAG AAC TGC AAG ATG AAG TTT GGC TCC TGG ACA TAT GAC AAG GCC TAT GAT CAG CAG ATG GAG AGG ACA GTG GAC CTG
 Asp Gln Gln Asn Cys Lys Met 150 Lys Phe Gly Ser Trp Thr Tyr Asp Lys Ala 160 Lys ile Asp Leu Glu Gln Met Glu Arg Thr Val Asp Leu
 540 AAG GAC TAC TGG GAG AGT GGC GAG Trp Ala ile ile Asn Ala Thr Gly Thr 570 TAT AAC AGT AAG AAG TAC GAC TGC TGC GCG GAG ATC TAC
 Lys Asp Tyr Trp Glu Ser Gly 180 Trp Ala ile ile Asn Ser Lys Lys Tyr Asp Cys Cys Ala 200 Glu ile Tyr
 630 CCC GAT GTC ACC TAC TTT GTC ATC CGC CGG CTG CCG CTG TTC TAT ACC 660 AAC CTC ATC ATC CCA TGC CTG CTC ATC ATC TGC CTC
 Pro Asp Val Thr Tyr Tyr Phe Val ile Arg Arg Leu Pro Leu Phe Tyr Thr 210 ile Asn Leu ile ile Pro Cys Leu Leu ile Ser Cys Leu
 720 ACT GTG CTC GTG TTC TAC CTG CCT Thr Val Leu Val Phe Tyr Leu 240 TCC GAG TGT GGA GAG AAG ATC ACG CTG TGC Cys ile Ser Val Leu Leu Ser Leu Thr Val 260 TTC CTG CTG
 810 CTC ATC ACG GAG ATC ATC CCG TCC ACC TCG CTG GTC ATC CCA CTC ATC GGC GAG Thr Ser Leu Val ile Pro Leu ile Gly 270 ACC GAG Thr Leu Ser
 840 CTC TCT

FIG. 18B

ATC GTT ATC ACA GTC TTC GTG CTC AAT GTA CAC CAC CGC TCC CCC AGC ACC ACC ATG CCC AAC TGG GTA AGG GTA GCC CTG CTA GGC
 Ile Val Ile Thr Val Phe Val Leu Asn Val His His Arg Ser Pro Ser Thr His Asn Met Pro Asn Trp Val Arg Val Ala 930 960
 300
 CGG GTG CCC AGG TGG CTG ATG ATG AAC CGG CCC CTG CCA CCT ATG GAG CTC CAT GGC TCC CCG GAT CTG AAG CTC AGC CCC TCA TAC CAT
 Arg Val Pro Arg Trp Leu Met Met Asn Arg Pro Leu Pro Pro Met Glu Leu His Gly Ser Pro Asp Leu Lys Leu Ser Pro Cys Val 1020 1050
 330
 TGG CTA GAG ACT AAC ATG GAT GCT GGA GAA AGG GAG GAG ACA GAG GAA GAG GAA GAA GAT GAA AAC ATA TGT GTG TGT GCA GGC
 Trp Leu Glu Thr Asn Met Asp Ala Gly Glu Arg Glu Glu Thr Glu Glu Glu Glu Asp Glu Asn Ile Cys Val Cys Val 1100 1140
 360
 CTT CCA GAC TCT TCG ATG GGT GTC CTC TAT GGC CAT GGC GGC CTG CAT CTG AGA GCC ATG GAG CCT GAG ACC AAG ACT CCA TCC CAG GCT
 Leu Pro Asp Ser Ser Met Gly Val Leu Tyr Gly His His Gly Gly Leu His Leu Arg Ala Met Glu Pro Glu Thr Lys Thr Pro Ser Gln Ala 1200 1230
 390
 AGC GAG ATT CTG TCA CCT CAA ATA CAG AAA GCA CTA GAA GGT GTA CAC TAC ATT GCT GAC CGT CTG AGG TCT GAG GAT GCT GAC TCT
 Ser Glu Ile Leu Leu Ser Pro Gln Ile Gln Lys Ala Leu Glu Gly Val His Tyr Ile Ala Asp Arg Leu Arg Ser Glu Asp Ala Asp Ser 1290 1320
 420
 TCG gtgaat.....ctaacttcag GTG AAG GAA GAC IGG AAG TAT GTG GCC ATG GTG GTA GAC CGG ATA TTC CTC IGG CTG TTC ATT ATC
 Ser Val Lys Glu Asp Trp Lys Val Tyr Val Ala Met Val Val Asp Arg Ile Phe Leu Trp Leu Phe Ile Ile 1350 1380
 450
 GTC TCG TTC CTG GGG ACC ATC GGA CTC TTC CTT CCT CCA TTC CTG GCT GGA ATG ATC TAA CTTCATGTCCTTCATGTTGGCTCCAGGTGGCCTTCGTA
 Val Cys Phe Leu Gly Thr Ile Gly Leu Phe Leu Pro Pro Phe Leu Ala Gly Met Ile 1410 1440
 470
 ACTATCTTCTAGTCTCTGATGGAGCCATCTCTAGATACTCTTTTGAC.....3'
 1500

FIG. 18C

[illegible]

SIGNAL PEPTIDE

[illegible]

	NEURANE SPANNING I										NEURANE SPANNING II										NEURANE SPANNING III																																																																															
01	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

MEMBRANE SPANNING III

10TITTHIWFSTUXRPSRVOEKRIFTEDIDISDISOXPPGPPMGFH
 11ALPHA1
 12GRVQHWLWUJRLPLPMELNGSPOLKLSPSYHWLETHUDAGEEREETEEREEEDENICVCAGLPOSSGVLVGHGGLHLRAMEPETKTPSOA
 13ALPHA2
 14LMLRYVFNTRPTSGEDTPTKTFYGAELSNLWCFSRADSKCEGPPCDDGTGCHHPRVKISNFSAHLTRSSSESVNAYL
 15ALPHA3
 16LDIVQRLLFUKRPSVYKQDNCRRLLJESWIKMAHAPRFWPEPGEFGLISDICHNOGLSPARTFCNPTDTAVETOPTCRSPPLEVPOLKTSVEKASPCPSGSCPPPKSSSGAPHLIKK
 17ALPHA4
 18CYTOPLASMIC REGION

CYTOPLASMIC REGION

ALPHA1
ALPHA2
ALPHA3
ALPHA4

MSLVHVNPSDSOEAEDGIRCRSHS IDVCVSDGGAASLADSKPTSSPISLKARFSOLAVSDOASPCKCTCKEAPSPVSPVTVLKAGGTKAPPHLPLSALTRAVEGSDVLAHILKAE
CYTOPLASMIC REGION

AMPHIPHATIC HELIX

SEILLSNDIOXKALEGPHIAORLSE
SLALSNDIKERIOSXIAENHUKAO
SPLIKHNEVKSQIEGKIEETUKSD

CYTOPLASMIC REGION

	1	2	3	4
QESHNAAE	EE	EE	EE	EE
DADSSVKE	DM	DM	DM	DM
HYAKEI	OD	DM	DM	DM
DIDFSVKE	DM	DM	DM	DM

MEMBER SPRING IV

SPLIKHNEVXSMIEGXTIETUKSD
 SEILLSIOIOKALEGHIADRLHSE
 SLALSNEIKKAIOSXIAEIMKAO
 OHLPLSPALTRVEGVJLLOHLKAE
 AMPHIPATHIC HELIX

FIG. 19

FIG. 20A

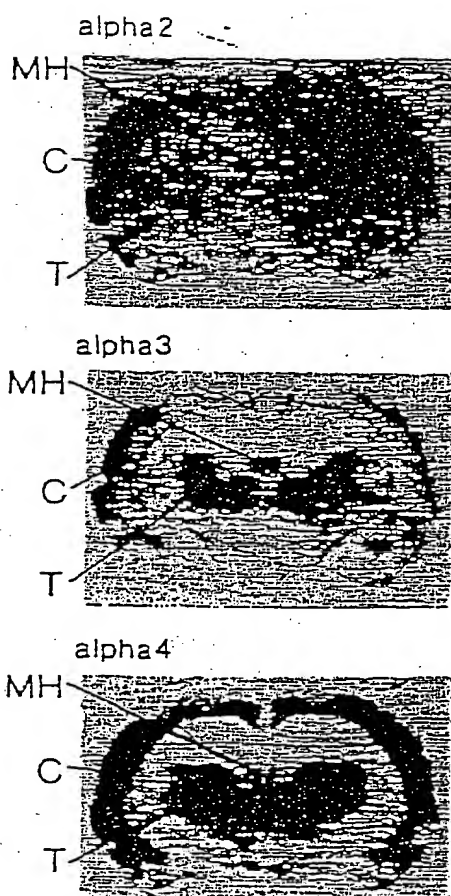
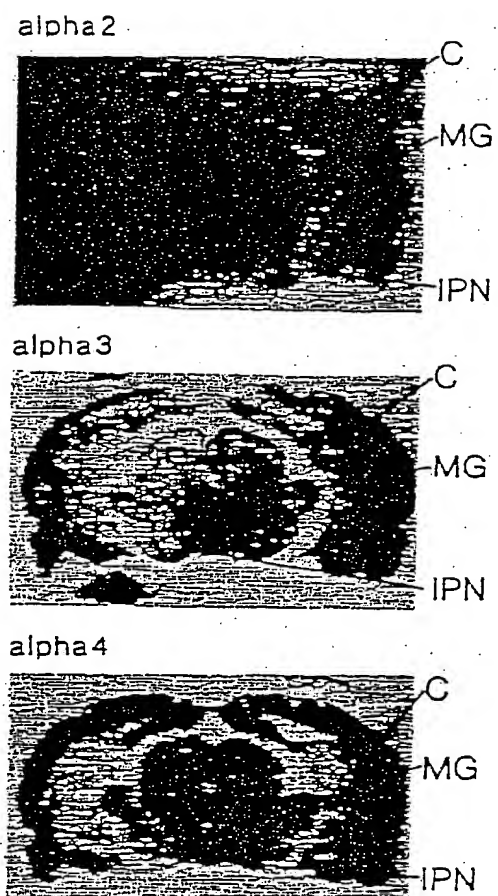


FIG. 20B



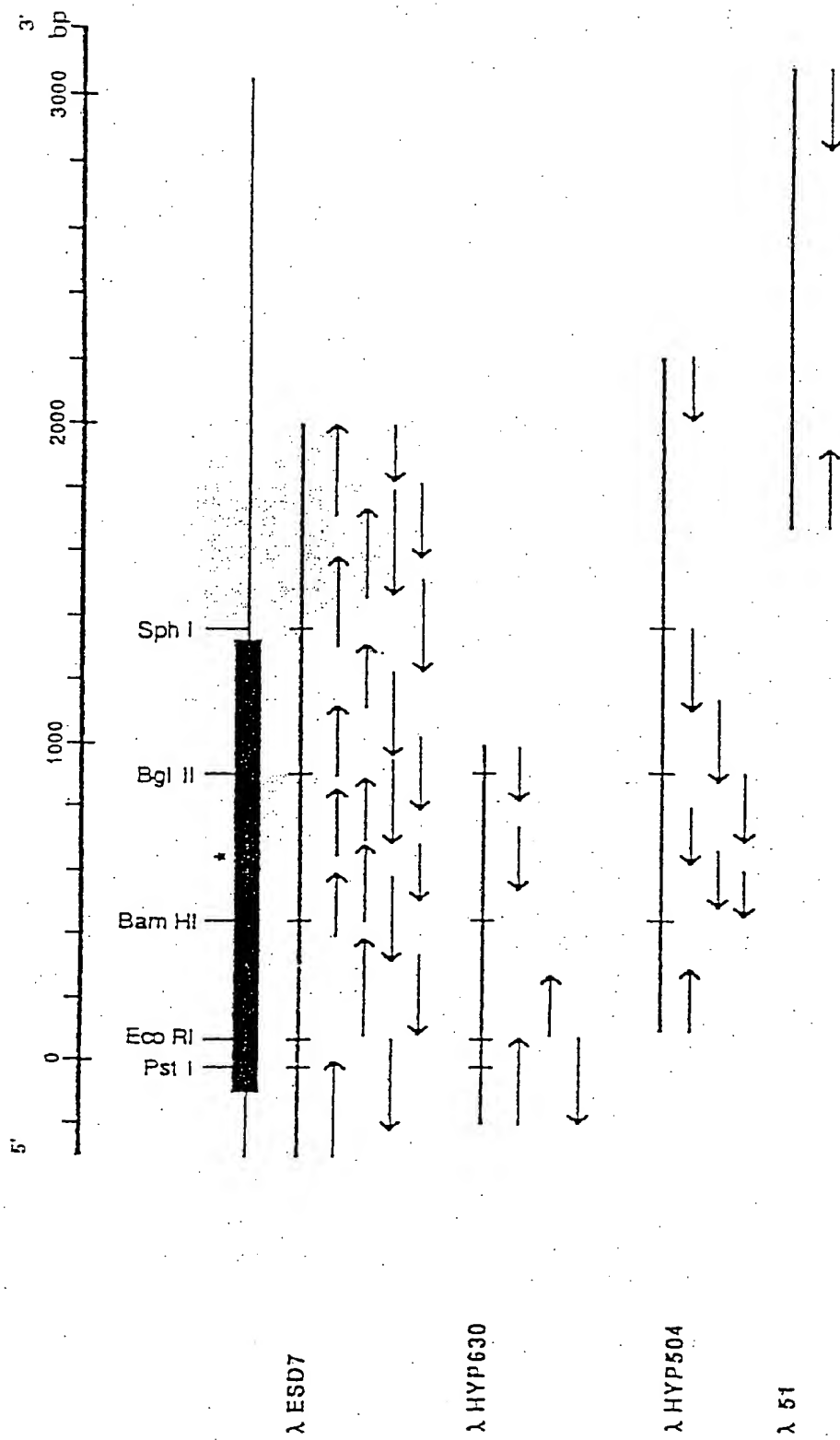


FIG. 21A

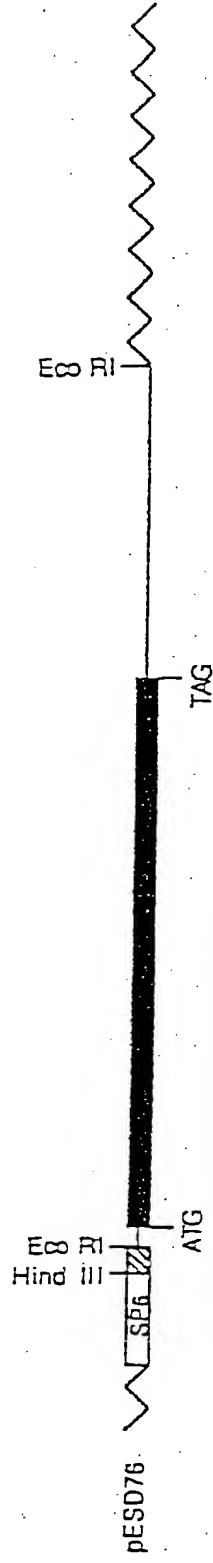


FIG. 21B

5'GACAGACATT

-240 -220 -200 -180 -160 -140

TGTGGTCATCAGCTGATTATTTTCATCAGGAGCTGGTCCCTGGTTCATCAGGCTTGAACCACTCACATTTTGTGTTTAAACCCCTGATCCTTCCAGTGGAAACACT

-120 -100 -80 -60 -40

CTGCGCTTCAAGGAAATGTCTCTCTGAAGCAGAGCTC Met Thr Gly Phe Leu Arg Val Phe Leu Val Leu Ser Ala Thr Leu Ser Gly Ser Trp Val

-20 -10 1 10 20 30 40 50

ACT CTT ACG GCC ACT GCA GGA CTC AGC TCA GTG GCT GAA CAC GAA GAC GAA CAC CAC CTC CTC AGA CAT TTG TTC CAA GGT TAC CAG AAA TGG GTC

-20 -10 1 10 20 30 40 50

Arg Pro Val Leu Asn Ser Ser Asp Ile Ile Lys Val Tyr Phe Gly Leu Lys Ile Ser Gln Leu Val Asp Val Asp Gln Lys Asn Gln Leu

80 90 100 110 120 130 140

CGC CCT GTG TTG AAT TCC AGT GAC ATC ATA AAA GTG TAT TTT GGA TTA AAA ATA TCC CAG CTT GTG GAT GTG GAA AAG AAT CAG CTG

160 170 180 190 200 210 220

Met Thr Thr Asn Val Trp Leu Lys Gln Glu Trp Thr Asp Gln Lys Leu Arg Trp Asn Pro Glu Glu Tyr Gly Gly Ile Asn Ser Ile Lys

240 250 260 270 280 290 300

ATG ACG ACA AAT GTG TGG CTG AAG CAG GAA TGG ACA GAC CAA AAA TTA CGC TGG AAT CCG GAA GAA TAT GGT GGA ATT AAT TCG ATA AAG

320 330 340 350 360 370 380

Val Pro Ser Glu Ser Leu Trp Leu Pro Asp Ile Val Leu Phe Glu Asn Ala Asp Gly Arg Phe Glu Gly Ser Leu Met Thr Lys Ala Ile

400 410 420 430 440 450 460

GTT CCA TCA GAA TCG CTC TGG CTG CCG GAC ATA GTT CTC TTT GAA AAT GCT GAC GCA CGT TTT GAG GGC TCC CTC ATG ACC AAG GCC ATT

480 490 500 510 520 530 540

Val Lys Ser Ser Gly Thr Val Ser Trp Thr Pro Pro Ala Ser Tyr Lys Ser Ser Cys Thr Met Asp Val Thr Phe Phe Pro Phe Asp Arg

560 570 580 590 600 610 620

GTG AAG TCC AGT GCA ACC GTC AGC TGG ACT CCT CCC GCC AGC TAC AAG AGT TCC TGC ACC ATG GAT GTC ACA TTT TTC CCG TTC GAC AGG

FIG. 22A

150 Gln Asn Cys Ser Met Lys Phe Gly Ser Thr Tyr Asp Gly Thr Met Val Asp Leu Ile Asn Glu Asn Val Asp Arg Lys Asp 170
 CAG AAC TGC TCG ATG AAG TTT GGA TCC TGG ACT TAC GAC GGT ACC ATG ATG GTT GAC CTC ATT CTA ATC AAT GAA AAC GAT GAC CGG AAA GAC 500
 440 460

180 Phe Phe Asp Asn Gly Glu Trp Glu Ile Leu Asn Ala Lys Gly Met Lys Gly Asn Arg Arg Glu Gly Phe Tyr Ser Tyr Pro Phe Val Thr 200
 TTT TTT GAT AAC GGA GAG TGG GAG ATA CTC AAC GCA AAG GGG ATG AAG GGC AAC AGA AGA GAA GGC TTT TAC TCC TAT CCG TTT GTT ACC 500
 520 540 560

210 Tyr Ser Phe Val Leu Arg Arg Leu Pro Leu Phe Tyr Thr Leu Phe Leu Ile Ile Pro Cys Leu Gly Leu Ser Phe Leu Thr Val Leu Val 230
 TAC TCT TTT GTC CTG AGA CGC CTG CCC TTG TTT TAC ACC CTC TTT TTG ATA ATC CCC TGC CTG GGG TTG TCT TTT CTC ACG GTC CTG GTG 680
 620 640 *

240 Phe Tyr Leu Pro Ser Asp Glu Gly Glu Lys Leu Ser Leu Ser Thr Ser Val Leu Val Ser Leu Thr Val Phe Leu Val Ile Glu Glu 260
 TTC TAC CTA CCC TCG GAC GAA GGG GAA AAA CTC TCA TTA TCC ACC TCC GGT TTG GTC TCT TTG ACG GTG TTT CTT TTA GTG ATT GAA GAA 700
 720 740

270 Ile Ile Pro Ser Ser Lys Val Ile Pro Leu Ile Gly Glu Tyr Leu Leu Phe Ile Met Ile Phe Val Thr Leu Ser Ile Ile Val Thr 290
 ATA ATC CCG TCC TCT TCG AAG GTC ATC CCC CTC ATT GGC GAG TAC CTC CTC TTC ATT ATG ATT TTT GTC ACG CTG TCT ATT ATC GTC ACG 820
 800 840 860

300 Val Phe Val Ile Asn Val His His Arg Ser Ser Ser Thr Tyr His Pro Met Ala Pro Trp Val Lys Arg Leu Phe Leu Gln Arg Leu Pro 320
 GGT TTT CTA ATT AAT GTC CAC CAC CAC AGA TCT TCC TCA ACG TAC CAT CCC ATG GCC CCC TGG GTG AAG AGG CTG TTT CTA CAA AGA CTC CCG 940
 880 900 920 960

330 Arg Trp Leu Cys Met Lys Asp Pro Met Asp Arg Phe Ser Phe Pro Asp Gly Lys Glu Ser Asp Thr Ala Val Arg Gly Lys Val Ser Gly 350
 ACA TGG CTT TGC ATG AAG GAC CCC ATG GAC CGC TTC TCT TTC CCG GAT GCA AAG GAG AGT CAT ACA GCC GTG AGG GGG AAA GTC TCA GGC 1040
 980 1000 1020

FIG. 22B

Lys Arg Lys Gln Thr Pro Ala Ser Asp Gly Glu Arg Val Leu Val Ala Phe Leu Glu Lys Ala Ser Glu Ser Ile Arg Tyr Ile Ser Arg 360 380
 AAA AGG AAA CAG ACT CCC GCC AGC GAT GGA GAA AGA GAT CTG GTC GCT TTC CTC GAG AAG GCC TCC GAG TCC ATC AGA TAC ATT TCG AGG 1080 1140
 1060 1100 1120
 His Val Lys Lys Glu His Phe Ile Ser Gln Val Val Gln Asp Trp Lys Phe Val Ala Gln Val Leu Asp Arg Ile Phe Leu Trp Leu Phe 400 410
 CAT CTG AAA AAG GAA CAC TTC ATC AGC CAG GTA GTG CAA GAC TGG AAA TTT GTG GCT CAA GTT CTG GAC CGC ATC TTC CTG TGG CTC TTT 1160 1220
 1180 1200
 Leu Ile Ala Ser Val Leu Gly Ser Ile Leu Ile Phe Ile Pro Ala Leu Lys Het Trp Ile His Arg Phe His 420 430
 CTG ATA GCT TCT GTG TTG GGT TCC ATT CTG ATT TTT ATT CCA GCC TTG AAG ATG TGG ATA CAT CGT TTC CAC TAG GAGCCACTCTCTGGACCCA 1240 1300
 1260 1280 1320
 TTTAGAGACATACATAGAGACAAATCCACCTTAGGACTGACAGCGGCTGGCATGTCTGACAGGAGCAGCCATGCAATGCTAGTGGTGGCCTTGTCTGTCTGGAGCTTCTGTG 1340 1400 1440
 1360 1380 1420
 ATTGCAGGGCACTGAGAGAAATCTGGGTTTGAGTTAGTGAGATGGTGGCTGCCATTAGAGAGGTGTAGTTGGGCAATTGGAGAGCGTCTCCATGTTATATTGTTATGTGGAGTTCTCTGAAC 1460 1520 1560
 1480 1500 1540 1580
 TACTCCCTCTGCTCATCCCTGAACGCACCTGGGGCTATGTGGTATTCTCTAGCAGTGTGGTGAACGCATTTTGACAATAAGTTTTCAGGAAATTACGGCAGGTACAACTCTCCACACACAGG 1600 1660 1680
 1580 1600 1620 1640 1660 1680 1700 1720 1740 1760 1780 1800
 TCAAATTTGCCACTTGTCAACGAGTGTCCCAAAATAGGGTCATTGAAGATGACCTTGAAATGGCTATGACAGATTCTCTTAAGCAGGTGTACTGGGAAGTTTGGCTCACTGACCTGGGAAC 1700 1760 1800
 1720 1740 1760 1780 1800
 TTTTCGAATGCAGGTAGGAACCTGGGGTCATTTCTAGCTTGTCTGTAAGTTTCCATCAAAATAAAGTTTACCCCGAGAAACAGCCATTCCCTAGTAGAAGCTGTATTTTTCACACACATCTC 1820 1880 1900
 1800 1820 1840 1860 1880 1900
 TTTTTTTCCC.....J'
 1930

FIG. 22C

NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS

BETA 3	UTGFLRVFLVLSATLSGWSVTLTATAGLSVAZPVEDALLRHLFOGQKWWHLVLSGDIKVVYFGLKISQVYQKHNQTNVYALKDEMTDOXRNHINVEEYGGI
BETA 2	MLACHAGHSHNALFSFLLWLCGSLGDTIGERLVEHLLDPNRKLLIMATIGSELVTOLVSLACLISLHIREQNTONMLTDEEYRITKCKEDFDNH
ALPHA 2	MTLSHALOFNTHLYLWCLLVPAVLTOOGSHTHLDRLEKH LFGCNRWAVNVPVTSOWVIFGSLIATIDVQNDQTNVYALKDEMTDOXRNHINVEEYGGI
ALPHA 3	HGVLLPPPLSLMLVLMPLAASASEASHRLFOY LFEDNEIIRGVANSHPIIOFEVMSLQVYKQDQVNDDEIDQLKQINQYKCKKSDYOGV
ALPHA 4	MEIGGPGAPPLLLPLLLLTGTLGPASSSHIETRAHAEHLKRLFGCNRWHLVLSGDIKVVYFGLKISQVYQKHNQTNVYALKDEMTDOXRNHINVEEYGGI
	-----SIGNAL PEPTIDE----->
BETA 3	HSIKVPSSESLQDDVTENADRRFEGSLTKKAIKVSSTVSTTPASINSSSTUDTFFPTDRQNLSSWTKGRTYDGTUVDVILINENVDKDKDFDITWEIHAKEG
BETA 2	KKVRLPSKHIIQADVVLYIADGAYEVSFSIAVSYDGSIFMLPAIYKSAKIEVAFEPDQNGTNTKPRQNTDATEIDVVKSDVASLDOFTPSGLQDIALP
ALPHA 2	TSLRVPSSEMLQDDVTENADRRFEGSLTKKAIKVSSTVSTTPASINSSSTUDTFFPTDRQNLSSWTKGRTYDGTUVDVILINENVDKDKDFDITWEIHAKEG
ALPHA 3	EFNRVDAEKIKKDDVTENADRRFEGSLTKKAIKVSSTVSTTPASINSSSTUDTFFPTDRQNLSSWTKGRTYDGTUVDVILINENVDKDKDFDITWEIHAKEG
ALPHA 4	TSIRIIPSELINDDVTENADRRFEGSLTKKAIKVSSTVSTTPASINSSSTUDTFFPTDRQNLSSWTKGRTYDGTUVDVILINENVDKDKDFDITWEIHAKEG
BETA 3	MKGRRR EGFYS VPFTVTSFVLRLPLETILFLIPGGLSFTTVNVLPSDEGKLSSTSVLSLVLLVITVEEIISSSKVITTEIIFLQIFVLSTIV
BETA 2	RRHEHPDDSTVDVITDFIIRKXPLEVTINLIPGGLSFTTVNVLPSDEGKLSSTSVLSLVLLVITVEEIISSSKVITTEIIFLQIFVLSTIV
ALPHA 2	TVNSKKYDCCAEI VPDVLYFVIRRLPLEVTINLIPGGLSFTTVNVLPSDEGKLSSTSVLSLVLLVITVEEIISSSKVITTEIIFLQIFVLSTIV
ALPHA 3	YKHEIKYNGCEEI VODIIVSLYIRRLPLEVTINLIPGGLSFTTVNVLPSDEGKLSSTSVLSLVLLVITVEEIISSSKVITTEIIFLQIFVLSTIV
ALPHA 4	TYHTRKYECCEI VPDIIAFIRRLPLEVTINLIPGGLSFTTVNVLPSDEGKLSSTSVLSLVLLVITVEEIISSSKVITTEIIFLQIFVLSTIV
	-----TMD I----->
BETA 3	TVFVIRVHHSSTVYVPJAPVKKRLF ORLDRWL CUKDPHDFE SFPDGG
BETA 2	SVCLVVIHHSPTTDTAPVKKVFE EKLDITLLFLOOPRHQCARORLRLRROREREGEAVFFREGPAADPTCTCFVNP
ALPHA 2	TVFVIRVHHSPTTDTAPVKKVFE EKLDITLLFLOOPRHQCARORLRLRROREREGEAVFFREGPAADPTCTCFVNP
ALPHA 3	TVFVIRVHHSPTTDTAPVKKVFE EKLDITLLFLOOPRHQCARORLRLRROREREGEAVFFREGPAADPTCTCFVNP
ALPHA 4	TVFVIRVHHSPTTDTAPVKKVFE EKLDITLLFLOOPRHQCARORLRLRROREREGEAVFFREGPAADPTCTCFVNP
	-----TMD II----->
BETA 3	PLEVPDLKTSVEKASCPSPGSGPPKSSSGAPMLIKARLSVQHVPPSSOEAEDGIRCBRSRIQYCVSODGAASLADSKPTSSPTSLKARP SOLPVSDOASPCXCT
BETA 2	PLEVPDLKTSVEKASCPSPGSGPPKSSSGAPMLIKARLSVQHVPPSSOEAEDGIRCBRSRIQYCVSODGAASLADSKPTSSPTSLKARP SOLPVSDOASPCXCT
ALPHA 2	PLEVPDLKTSVEKASCPSPGSGPPKSSSGAPMLIKARLSVQHVPPSSOEAEDGIRCBRSRIQYCVSODGAASLADSKPTSSPTSLKARP SOLPVSDOASPCXCT
ALPHA 3	PLEVPDLKTSVEKASCPSPGSGPPKSSSGAPMLIKARLSVQHVPPSSOEAEDGIRCBRSRIQYCVSODGAASLADSKPTSSPTSLKARP SOLPVSDOASPCXCT
ALPHA 4	PLEVPDLKTSVEKASCPSPGSGPPKSSSGAPMLIKARLSVQHVPPSSOEAEDGIRCBRSRIQYCVSODGAASLADSKPTSSPTSLKARP SOLPVSDOASPCXCT
	-----CYTOPLASMIC REGION----->
BETA 3	ESDTAVRGKVSQKRNKOTP ASDGERVLVAFLEKASESIRYISRHVKKENHFIQVVOQWKEFAQVDRHFWLFIASVLSILIPALKWUWHIRFH
BETA 2	ASVOGLAGAFRAEPTAAG PGRSYGPGSCGLREAVDGVRFADHURSEDODSVREDNKYVAVQIDRFLVITVFCVFGTGVGMQOPLFONYTATTFLHPDHSAPSSK
ALPHA 2	YGHGGLHLRAUEPETKP SOASEILLSPQIOKALEGVHYADRLRSEDODSVREDNKYVAVQIDRFLVITVFCVFGTGVGMQOPLFONYTATTFLHPDHSAPSSK
ALPHA 3	ISHFSANLTHSSSSSESVH AVLSLSALSPEIKENIOSVKYIAEIKAKAOWHAKIEODQNSYVAVQIDRFLVITVFCVFGTGVGMQOPLFONYTATTFLHPDHSAPSSK
ALPHA 4	CKEPSVPSPVTLKAGGTAKAPPDHLPLSPALTRAVEGVQYADHLKAEDTDFSVKEENKYVAVQIDRFLVITVFCVFGTGVGMQOPLFONYTATTFLHPDHSAPSSK
	-----TMD IV----->

FIG. 23

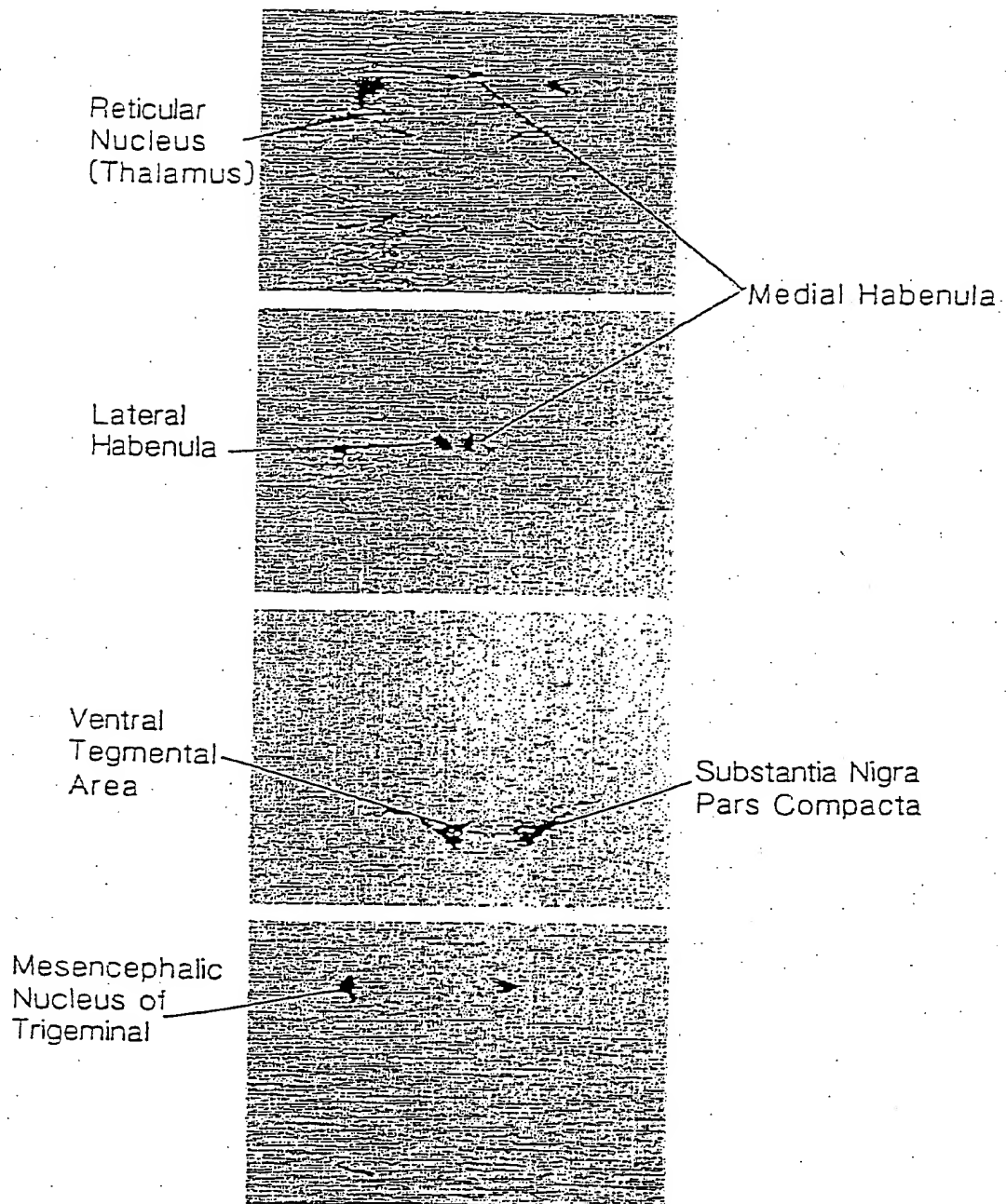


FIG. 24



FIG. 25

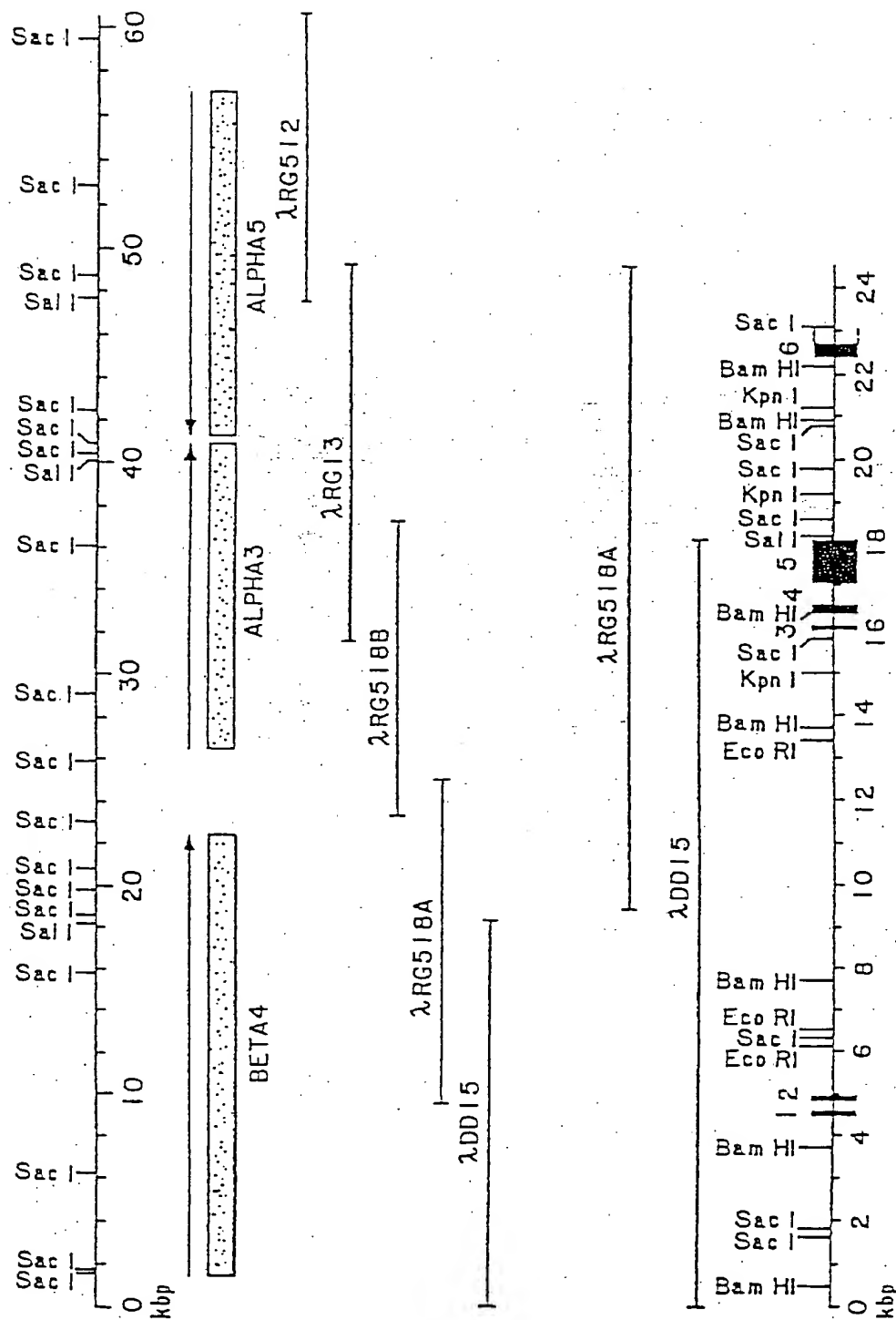


FIG. 26

5'...GCCAACCGGGACAT
-120

ACGCTACTGGCCTTCCATTGTAGAGTGACCGGCTGCCACCGGCTGGCC
-100
Met Arg Gly Thr Pro Leu Leu Val Ser Leu Phe Ser Leu Leu Gln Asp
-20
-40
-60
-80
-100

Gly Asp Cys Arg Leu Ala Asn Ala Glu Glu Lys Leu Met Asp Asp Leu Leu Asn Lys Thr Arg Tyr Asn Asn Leu Ile Arg Pro
-1
+1
1
20
40
60

Ala Thr Ser Ser Ser Gln Leu Ile Ser Ile Arg Leu Glu Leu Ser Ser Gln Leu Ile Ser Val
30
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500

Arg Glu Gln Ile Met Thr Thr Ser Ile Trp Leu Lys Gln
50
100
150
200
250
300
350
400
450
500

Ser Ser Cys Tyr Glu Gly Val Asn Ile Leu Arg Ile Pro Ala Lys Arg Val Trp Leu Pro Asp Ile Val Leu Tyr Asn As
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500

...cctaccccag T GCC GAT GCC ACC ACC TAT GAG GTG TCT GTC TAC ACC ACC AAC CTG ATT GTG CGT TCC AAC GGC AGC ATC CAG TGG CTG CCC CCT
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500

FIG. 27A

130 Ala Ile Tyr Lys Ser Ala Cys Lys Ile Glu Val Lys His Phe Pro Phe Asp Gln Gln Asn Cys Thr Leu Lys Phe Arg Ser Trp Thr Tyr
 CCT ATC TAC AAG AGT GCC TGC AAG ATT GAG GTG AAG CAC TTT CCC TTC GAC CAG CAG CAG AAC TGC ACC CTC AAA TTC CGC TCC TGG ACC TAT
 380 400 420 440

160 Asp His Thr Glu Ile Asp Met Val Leu Lys Ser Ala Thr Ala Ile Met Asp Phe Thr Pro Ser Gly Glu Trp Asp Ile Val Ala Leu
 GAC CAC ACG GAG ATT GAC ATG GTT CTT AAG TCG CCC ACG GCC ATC ATG CAT GAC TTC ACC CCC AGT GGT GAA TGG GAC ATT GTG GCC CTC
 480 500 520 540

190 Pro Gly Arg Arg Thr Val Asn Pro Gln Asp Pro Ser Tyr Val Asp Val Thr Tyr Asp Phe Ile Ile Lys Arg Lys Pro Leu Phe Tyr Thr
 CCA GGA CGG ACG ACG GTG AAC CCT CAG GAC CAC CCC ACG TAC TCG GAC GTG ACC TAT GAC TTC ATC ATC AAG CGC AAC GCG CTC TTC TAC ACC
 560 580 600 620

220 Ile Asn Leu Ile Ile Pro Cys Val Leu Ile Thr Ser Leu Ala Ile Leu Val Phe Tyr Leu Pro Ser Asp Cys Gly Glu Lys Met Thr Leu
 ATC AAT CTT ATC ATT CCT TGT GTG CTC ATC ACC TCG CTG GCT ATC CTG GTC TTC TAC CTG CCC TCC GAC TGT GGG GAG AAG ATG ACG CTC
 640 660 680 700 720

250 Cys Ile Ser Val Leu Leu Ala Leu Thr Phe Phe Leu Leu Leu Ile Ser Lys Ile Val Pro Pro Thr Ser Leu Asp Ile Pro Leu Ile Gly
 TGC ATC TCT GTG CTG CTG GCA CTC ACG TTC TTC CTG CTG CTC ATC TCC AAG ATC GTG CCT CCC ACC TCC CTT GAC ATA CCG CTC ATT GGC
 740 760 780 800

280 Lys Tyr Leu Leu Phe Thr Met Val Leu Val Thr Phe Ser Ile Val Thr Thr Val Cys Val Leu Asn Val His His CAC CAC CCC TCA CCC AGC ACT
 AAG TAC CTC TTG TTC ACC ATG GTG GAG TGC CTC GTC ACC TTT TCC ATG GTC ACC ACT GTG TGT GTC CTC AAT GTG CAC CAC CAC CCC TCA CCC AGC ACT
 820 840 860 880 900

310 His Thr Met Ala Ser Trp Val Lys Glu Cys Phe Leu His Lys Lys Leu Pro Thr Phe Leu Phe Met Lys Arg Pro Gly Leu Glu Val Ser Leu
 CAC ACC ATG GCA TCC TGG GTC AAG GAG TGC TTC CTG CAC AAA CAC CTG CCC ACC TTC CTC TTC ATG AAG CGT CCC GGT CTT GAA GTC AGC CTC
 920 940 960 980 1000

FIG. 27B

```

340
Val Arg Val Pro His Pro Ser Gln Leu His Leu Ala Thr Ala Asp Thr Ala Ala Thr Ser Ala Leu Gly Pro Thr Ser Pro Ser Asn Leu
360
3TC AGG GTC CCT CAT CCC AGC CAG CTG CAC TTG GCC ACA GCT GAT ACT GCA GCC ACC TCT GCC TTA GGC CCC ACC AGC CCA TCC AAC CTC
1020
1060
1080

370
Tyr Gly Ser Ser Met Tyr Phe Val Asn Pro Val Pro Ala Ala Pro Lys Ser Ala Val Ser Ser His Thr Ala Gly Leu Pro Arg Asp Ala
390
TAT GGG AGT TCC ATG TAC TTT GTG AAC CCT GTC CCT GCT CCT GCT GCT TCT GCA GTC AGC TCC CAC ACA GCA GGC CTC CCC AGG GAT GCC
1100
1120
1140
1160

400
Arg Leu Arg Ser Ser Gly Arg Phe Arg Glu Asp Leu Gln Glu Ala Leu Glu Gly Val Ser Phe Ile Ala Gln His Leu Glu Ser Asp Asp
420
CGT CTG AGG TCC TCC GGG AGG TTC CCG GAA GAT CTA CAG GAA GCA TTA GAG GGT GTC AGC TTC ATC GCC CAG CAT CTG GAG AGC GAT GAC
1200
1220
1240
1260

430
Arg Asp Gln Ser
CGA GAT CAA AGT gtagtcactg... Val Ile Glu Asp Trp Lys Phe Val Ala Met Val Val Asp Arg Leu Phe Leu Trp
1280
1300
1320

450
Val Phe Val Phe Val Cys Ile Leu Gly Thr Met Gly Leu Phe Leu Pro Pro Leu Phe Gln Ile His Ala Pro Ser Lys Asp Ser
470
GTG TTC GTG TTT GTG TGT ATT CTG GGC ACC ATG GGG CTC TTC CTG CCA CCC CTT TTC CAG ATC CAC GCA CCC TCC AAG GAC TCC TAG GCT
1340
1360
1380
1400
1420
1440
1460
1480
1500
1520

ACCGCGCTGTCTCGGHNCCGGGAAGTAGTGAGATGATATGAGAACGGGTGGGAGCGGGGTGTCTTTGGGGCTACCCGGCCCTGTCTCGGGCCCGGGGAAGTAGTGAGATGATATGA
1520

GAAGCGGTGGGAAGCAGCGGCGTGTCTTCGG...3'
1540

```

FIG. 27C

CGTTGCTCGGCGCTCGGGGTTATCCGCGAGGCGCGCTCGGCGGCGGCGATGGCGGCTCGGGGCGGCGGCTGCGCTGCTCTTG
-180 -160 -140 -120 -100

Met Val Gln Leu Leu Ala Gly Arg Trp Arg Pro Thr Gly Ala Arg Arg Gly Thr Ala Gly Gly Leu Pro Glu Leu Ser Ser Ala Ala Lys
ATG GTG CAG CAG CTG GCA GGG CGC TGG CGG CCG ACC GGG GCG CGG CGG ACC GCG CGG TGG CCT GAA CTA TCC TCT GCT GCC AAA
-80 -60 -40 -20 1

His Glu Asp Ser Leu Phe Arg Asp Leu Phe Glu Asp Tyr Glu Arg Trp Val Arg Pro Val Glu His Leu Ser Asp Lys Ile Lys
CAT GAA GAC AGC TTG TTT AGC GAT TTA TTT GAA GAC TAC GAA AGG TGG GTT CGC CCT GTG GAA CAC CTG AGC GAC AAG ATA AAA ATC AAG
10 20 30 40 50 60 70 80 90

Phe Gly Leu Ala Ile Ser Gln Leu Val Asp Val Asp Glu Lys Asn Gln Leu Met Thr Thr Asn Val Trp Leu Lys Gln Glu Trp Ile Asp
TTT GGC CTT GCG ATA TCT CAG TTA GTG GAT GTG GAT GAG AAA AAC CAG CTG ATG ATG ACG ACG AAC GTC TGG TGG AAG CAG GAA TGG ATA GAT
100 120 140 160 180

Val Lys Leu Arg Trp Asn Pro Asp Tyr Gly Gly Ile Lys Ile Ile Arg Val Pro Ser Asp Ser Leu Trp Ile Pro Asp Ile Val Leu
GTG AAA TTG AGA TGG AAT CCT GAC GAT TAT GGT GGG ATA AAG ATT ATA CGT GTT CCT TCG GAC TCC CTG TGG ATC CCA GAC ATC GTT TTG
200 220 240 260 280

Phe Asp Asn Ala Asp Gly Arg Phe Glu Gly Ala Ser Thr Lys Thr Val Val Arg Tyr Asn Gly Thr Val Thr Trp Thr Gln Thr Ala Asn
TTT GAT AAT GCA GAT GGA CGT TTT GAA GGG GCC AGC AGC AAA ACA GTT GTC AAG TAC AAC GGC ACT GTC ACG TGG ACG CAA TGG Pro Ala Asn
300 320 340 360

Tyr Lys Ser Ser Cys Thr Ile Asp Val Thr Phe Phe Pro Phe Asp Leu Gln Asn Cys Ser Met Lys Phe Gly Ser Trp Thr Tyr Asp Gly
TAC AAA AGT TCT TGC ACC ATC GAC GTT ACC TTT TTC CCG TTT GAT CTC CAA AAT TGT TCC ATG AAA TTC GGC TCG TGG ACA TAC GAT GGA
380 400 420 440 460

Ser Gln Val Asp Ile Ile Leu Glu Asp Gln Asp Val Asp Arg Thr Asp Phe Phe Asp Phe Asp Asn Gly Glu Trp Glu Ile Met Ser Ala Met Gly
TCC CAG GTT GAT ATA ATC CTA GAG GAC CAA GAT GTC GAC AGA ACA GAC TTT TTC GAC AAT GGA GAG TGG GAA ATC ATG AGC GCG ATG GGG
480 500 520 540 560

Ser Lys Gly Asn Arg Thr Asp Ser Cys Cys Trp Tyr Pro Tyr Ile Thr Tyr Ser Phe Val Ile Lys Arg Leu Pro Leu Phe Tyr Thr Leu
AGC AAG GGG ANC CCG ACG GAC AGC Ser Cys Cys Trp Tyr Pro Tyr Ile Thr Tyr Ser Phe Val Ile Lys Arg Leu Pro Leu Phe Tyr Thr Leu
580 600 620

FIG. 28A

220 Phe Leu Ile Ile Pro Cys Ile Gly Thr Ser Phe Phe Leu Thr Val Val Val Phe Tyr Leu Pro Ser Asn Glu Gly Glu Lys Ile Ser Leu Cys
 TTT CTT ATT ATC CCC TGC ATT GGG CTC TCA TTT TTT CTG ACT CTG GTT GTT GTC TTC TAT CTC CCT TCA AAC GAG GGT GAA AAG ATT 720
 640
 250 Thr Ser Val Leu Val Ser Leu Thr Val Phe Leu Leu Val Ile Glu Glu Ile Ile Pro Ser Ser Lys Val Ile Pro Leu Ile Gly Glu
 ACC TCA GTG CTC GTG TCT CTG ACT GTC TTC CTT TTT TTT GTA ATC GAA GAG ATC ATA CCA TCA TCT TCC AAA GTC ATA CCC CTG ATT GGG GAG
 740
 280 Tyr Leu Val Phe Thr Met Ile Phe Thr Leu Ser Ile Met Val Thr Val Phe Ala Ile Asn Ile His Arg Ser Ser TCC TCC Thr His
 TAC TTG GTG GTG TTC ACC ACC CTA TCC ATT ATG GTG ACT GTC TTT GGC ATC AAC ATC CAC CAC CGC TCT TCC TCC ACA CAC
 820
 310 Asn Ala Met Ala Pro Trp Val Arg Lys Ile Phe Leu His Lys Leu Pro Lys Leu Thr Val Val Phe Leu Cys Met Arg Ser His Ala Asp Arg Tyr Phe Thr
 AAC GCT ATG GCG CCC TGG GTT CGT AAG ATA TTT CTC CAC CAC AAG CTT CCC AAG CTG CTC TGC ATG AGA AGT CAT GCG GAT AGG TAC TTC ACT
 920
 340 Gln Arg Glu Glu Ala Glu Ser Gly Arg Gly Pro Lys Ser Arg Asn Thr Leu Glu Ala Ala Leu Asp Cys Ile Arg Tyr Ile Thr Arg His
 CAG AGA GAA GAA GCC GAG AGT GGT GGA CCT AAA TCT CTC GCG AAC ACT TTG GAA GCC GCA Ala Leu Asp Cys Ile Arg Tyr Ile Thr Arg His
 1000
 370 Val Val Lys Glu Asn Asp Val Arg Glu Val Val Val Glu Asp Trp Lys Phe Ile Ala Gln Val Leu Asp Arg Met Phe Leu Trp Thr Phe Leu
 GTC GTG AAA GAG AAC GAC GTC CGC GAG GAG GTT GTT GAA GAT TGG AAA TTC ATA GCC CAA GTC CTT GAT CGG ATG TTT TTG TGG ACG TTT CTT
 1100
 400 Leu Val Ser Ile Ile Gly Thr Leu Gly Leu Phe Val Pro Val Ile Tyr Lys Trp Ala Asn Ile Ile Val Pro Val His Ile Gly Asn Thr
 CTG GTG TCA ATC ATT GGG ACT TTA GGG CTT TTT GTT CCT GTC ATT TAT TAT AAA TGG GCC AAT ATA ATA GTC CCA GTT CAC ATT GGA AAC ACA
 1200
 1260

FIG. 28B

ile Lys
 ATT AAG TGA AACCAAGAAATTACCCTGTGGATTAGTGAGCAGTCTATGCGAGCTCTTAGGACATGTATGCTGTTATGGAAATGTGAAGGTAGTTACAATTTGACATAGGCTATAACA
 1280 1300 1320 1340 1360 1380
 GATTAGCAATTTCTAACATTGGCTTAATGTTGTCCATTAGAACTGCAGTAATAACCTCAATAGCAACAACACATTGTCTGCCTGCACCTAGTGAAGGCCTAGCATCTGCATCCTGGCAA
 1400 1420 1440 1460 1480 1500
 ACCCTACCAATTTGCAACCAATGATGAAGGCCATCCTTGGAGTGTCTGGGAAACTCAACTGTATTGGAAGACTATTAAACTCCCCCAATTTAGTAGGAACATATATATGTGTGGTT
 1520 1540 1560 1580 1600 1620
 TTGAATTTTCAGAAATGGGTCTTTGGGCTTTGTTAAATTTGCTGGGCTAGCACAAACCTCCTGAGTAGCTGGGACCATGGGTGTGCTGCACCTTTGCCCTGTTCTGTATTCACAGATATA
 1640 1660 1680 1700 1720
 AATATACATCATTATTTATAGGAGGTAGGCCCAATTACTTGGGTTTAAATAACTTAAATGTCAGTTAGGTTTAAATTAACCTTATGTCAGGTAATGTTCTATTGCTGTGAMGAGACATC
 1760 1780 1800 1820 1840
 ATGACCATCMTCTTATTAAGAAACATTTTCATCAGTGTGCTGGCTTACAGTTTTTGAGGTTTAGCCAAATTATCACAGTGAAGCATGATAGCATCCAGGTAGACATATATGCTGGATCC
 1880 1900 1920 1940 1960
 AGGAGTTCTCTACATCTGGATCAGCAGGCGAGAGAGAGAGAGGCCACTGGACCTGGCTTGAGCATCTGAAACCTCAAGGCCACCTCCAGTGAACACACTTCCCCCAACAGGCC
 2000 2020 2040 2060 2080
 ACACCTCCTAATAGTGCCACTTGGCTGGTGAATCAAGCATTCAGTCTATGGGTCTACGAGAGGCCATTCCCTAATCAACCCACACTTAATAGGATGCTATTTCTTTACTGACATTTTAAATAAG
 2100 2120 2140 2160 2180 2200
 CGACAAATGGTAACTAGAAACATCGTAGGCCCACTTTACTCTTTTATATGTTAATGATTGGCTTTTATATTAAGTATTTTACAGGCTATCTGMAAACATGTAACAGGCAACTCCCTG
 2220 2240 2260 2280 2300 2320
 CAGACACATCTTTGTAAATGACTTTTAAATCCTGCAGTGGCATGTTCTGTTAATGACTTCAGTCTCCTGTCACACACTCCGGTTAGTAGAGCCATTTATGTACATCGCGTATCCCTG
 2340 2360 2380 2400 2420 2440
 ATTTACAGAGCAACTGTGCAGTTGCACAGGTTCCACCTCAAAATGGGATGCCATGACTCTGTCTGGATAAATCTGTGGAAACCACTTCTGAGCTGGATACGGTGCCTCATACCTGTCA
 2460 2480 2500 2520 2540 2560
 TGTCTACTTTACAGGAGGCGAGGCGGGAATTGCTGTGAGTTGTTGGTCAGGCTGGGTTACATATGAGACCCCTGTCTCAGAAACCAACAAACAACTTCCCCCTGTGAGTTGATATAG
 2580 2600 2620 2640 2660 2680
 CACACTGTCAATCCGAATTGGGGATCCTCTAGAGTCAAGCTTGGGCTAATCATGATGGTGCATAGCTGTTCTCTGTGTGMAATTGTTATCCG
 2700 2720 2740 2760 2780

FIG. 28C

B1TA2 HLACHAGHSHALFSLSLMLCSGLGDTTEERLVEHLLDPYRYHKLIRPATNGSELVTVQLHVSQAQLISVHEREQIHTHVMLTQEMEDYRLTWKPEDFDHMK
 B1TA3 HTGFLRVFLVLSATLSGSWVTLTATAGLSSVAEHEDALLRHLFOGYQXVRPVLHSSDIIKXVYFGLXISQLVDVEKNHQLHTHVMKQEWTDQKLRHNPPEEYGGIN
 B1TA4 HRTPLDLVLSLQOGDCRLANAEKLMDDLLNKTRYNNLIRPATSSQLISIRLELSQLISVNEREQIHTTSIMLKQEWTDYRLAHNSSCYEGVN
 ——— SIGNAL FIFTIDE ———

B1TA2 KVLPSXHIWLPDVLYHNDGHYEVSYSHAVVSYDGSIFWLPAPAIYKSACKIEVKHFFPDQONCTHMFRTSWTYORTEIDLVKSOVASLDOFTPSGEWDIIALPG
 B1TA3 SIKVPSESLWLPDIVLFEHADGRFEGSLMTKAIWKSSGTYSWTPPASYSKSSCTHDTVTFPPFDROMCSHKFGSWTVOGTHVDLILINERHVRKDFDFONGEWEILHAKG
 B1TA4 ILRIPAKRVMLPDIIVLYHNDGHYEVSVYTHIVRSNGSIQWLPAPAIYKSACKIEVKHFFPDQONCTLNFRTSWTYDHTEIOHVLKSATAINDDFTPSGEWDIIVALPG

B1TA2 RRHNPDDOS TYVDITYDPIIRRRKPLFYTHLIIPCVLITSLAILVFLYLPSCGEXMTLCISVLLALTFFLLLSKIVPPTSLDVPPLVGKYLHFTHVLVTFISVTSV
 B1TA3 HKGHRREGFYSPFVTVSVLRRRLPLFYTLIIIPCLGSLFTVLVFLYLPSCGEXMTLCISVLLALTFFLLLSKIVPPTSLDVPPLVGKYLHFTHVLVTFISVTSV
 B1TA4 RRTVNPQDP SYVDVTVYDPIIRRRKPLFYTHLIIIPCVLITSLAILVFLYLPSCGEXMTLCISVLLALTFFLLLSKIVPPTSLDVPPLVGKYLHFTHVLVTFISVTSV
 ——— MSR I ——— ——— MSR II ——— ——— MSR III ———

B1TA2 CVLHVHHRSPPT HTHAPWVKVVFLEKPLTLFLQOPNHRCARORLRLRRQREGEAVFFREGPAADPCSVGPCSCG
 B1TA3 FVIHVHHRSSSTYBPHAPWVKRLFLORLPRWLCHKDHPDRFSFPDGKESDTAVRGKVSXKOTPASDGERVLVAFLEK
 B1TA4 CVLHVHHRSPST HTHASHVKECFLHKLPTTLFHKRPGLEVSLVRVPHPSQLHATADTATASALGPTSPSNLYGSSHVYVNEVPAAPKSAVSSHTAGLPRDARLRS
 ———

B1TA2 LREAVDGVRFIADHHRSEDDOSVREDWKYVAHVIDRLFLHIFVFCVFGTVGMFLQPLFQWYTAITTLHPDHSAPSSK*
 B1TA3 ASESIRYISRHVKKEHFISQVQDNKFVAQVLDRIFLHLFLIASVLGSLIIFIPALKMHIHRFH*
 B1TA4 SGRFREDLQEALEGVSFIAQHLESDDRDOSVIEDHKTVAHVVDRLFLNVFVFCILGTHGLFLPLPLFOIHAPSKDS*
 ——— MSR IV ———

FIG. 29

ALPHA2	HTLSHALQFNTHLYLWCLLLVPAVLTOQGSHTHAEDRLFKHLFGGYNHRAVRPHTSDVIVRFGLSIAQLIDVDEKQNMHTTHVHLKQEWNDYKLRWDPAE
ALPHA3	HGVLLPPLSHLMLVHLMLPAASASEAEHRLFYLYFEDYNEIIRVANVSHRPIIQFVSHSQLVKVDEVHQTHEINHLKQIWHQYKLRKPKSPD
ALPHA4	HEIGGPGAPFPLLLPLLLGLTGLPASHSHIETRAHAEERLKRFLSGYHKWSRPVCHISDVVLVRFGLSIAQLIDVDEKQNMHTTHVVKQEWNDYKLRWDPGD
ALPHA5	HVQLLAGHWRPTGARRGTAGGLPELSAAKHEDSLFRDLFEDYERHVRPVEHLSDKIKIKTGLAISQLVDVDEKXQLGHTTHVHLKQEWIDVKLWHNPD
	----- SIGNAL PEPTIDE -----
ALPHA2	FGRVTSRVPSEHITHIPDIVLYNHADGEFAVTHNTKAHLFFGTGVHVPPIAYKSSCSIDVTFFPDQONCKMKFGSWTYDKAKIDLEQHENTVDLKDYMESGEWA
ALPHA3	YOGVEFRHVPKAEKIMPOIVLYNHADGDFQVDDTKALLKYTGVTHTPPAIFKSSCKIDVTFFPDQONCTHKFGSHSYDKAKIDLVLIGSSHMLKDYMESGEWA
ALPHA4	YENVTSIRIPSELIWRPOIVLYNHADGDFAVTHLTKAHLFYDGRVQHTPPAIIYKSSCSIDVTFFPDQONCTHKFGSWTYDKAKIDLVSINHRVDDQLDFMESGEMV
ALPHA5	YGGIKIIRVPSDSLHNPDIIVLFDNADGRFEGAS TKTVVRVNGTVTHTQTPANYKSSCTIDVTFFPDQONCTHKFGSWTYDGSQVDIILEDQDVDRDTDFDNGEWE
	----- ** -----
ALPHA2	IINATGTYNSKKYDCCAEIYVDVTVYVIRRLPLFYTIINLIIPCLLISCLTVLVYLPSECGEKITLCISVLLSLTVLFLLLITEIPSTSLVPLIGEYLLFTMHF
ALPHA3	IIKAPGYKHEIKYNCCEEIIYQDITYSLYIRRLPLFYTIINLIIPCLLISCLTVLVYLPSECGEKITLCISVLLSLTVLFLLLITEIPSTSLVPLIGEYLLFTMHF
ALPHA4	IYDAGTYNTRKYECCEAEIYVDITYAFIIRRLPLFYTIINLIIPCLLISCLTVLVYLPSECGEKITLCISVLLSLTVLFLLLITEIPSTSLVPLIGEYLLFTMHF
ALPHA5	IMSANGSKGHRDSCCH YPIITYSFVIKRLPLFYTLFLIIPCIGLSCLTVLVYLPSECGEKISLCTSVLSTVFLVLLVIEEIIIPSSSKVPLIGEYLVFTMHF
	----- MSR I ----- MSR II ----- MSR III -----
ALPHA2	VTLSIVITVFLVHVRHRSPTNNHPN WVRVALLGRVPRHLMHNRPLPPHELHGSDDLKLSPSYHMLETHMDAGEREREETEEEEEDENICVCAGLPDSSHGVLVG
ALPHA3	VTLSIVITVFLVHVRHRSPTNNHPT WVKAVFLMLLPVHVRHRSPTSGEDTPKTRTFYGAELSLNLCFSRADSKSKCEGYPCQDGTGCGYCHHRVRVKISHFSANL
ALPHA4	VTLSIVITVFLVHVRHRSPTNTHPA WVRVFLDIVPRLLEHHRPSVVDKNCRRLLIESHHKMANPRTHPEFVGEPCILSDICNOGLSPAPITFCNPTDTAVETQP
ALPHA5	VTLSIHVTVFAINIHRRSSSTHNAHAPVVRKIFLHKLPLKLLCHRSHADRYFTQREAEESGAGPKSRNT

ALPHA2	HGGLHLRAHEPETKTPSQA
ALPHA3	TRSSSESSEVNAVL
ALPHA4	TCRSPPLEVPDLXTSEVEKASPCSPGSCPPPKSSSGAPHLIKARSLSVQHVFPSSQEAADGIRCHRSRISQICVSDQGAASLADSKFTSSPTSLKARPSQLPVSDQ
ALPHA5	
ALPHA2	SZILLSPOIKALEGVHYIADRLRSEDADSSVKEDMKYVAHVVDRIFLWHFIIVCFGLGTIGLFLPFPFLAGHI*
ALPHA3	SLSALSPEIKEATQSVKYIAENHKAQHVAKEIQDDMKYVAHVVDRIFLWHFIIVCFGLGTIGLFLPFPFLAGHI*
ALPHA4	ASPCCKCTCKEPSVSPVTVLKAGGTAKAPPQHLPLSPALTRAVEGVQYIADHLKAEADTFSVKEDMKYVAHVVDRIFLWHFIIVCFGLGTIGLFLPFPFLAGHI*
ALPHA5	LEAALDCIRYITRHHVVKENDVREVVEDMKFIAQVLDHRLHFLMTFLVLSIIIGTLGLFVPVFLNGPI*
	----- MSR IV -----

FIG. 30

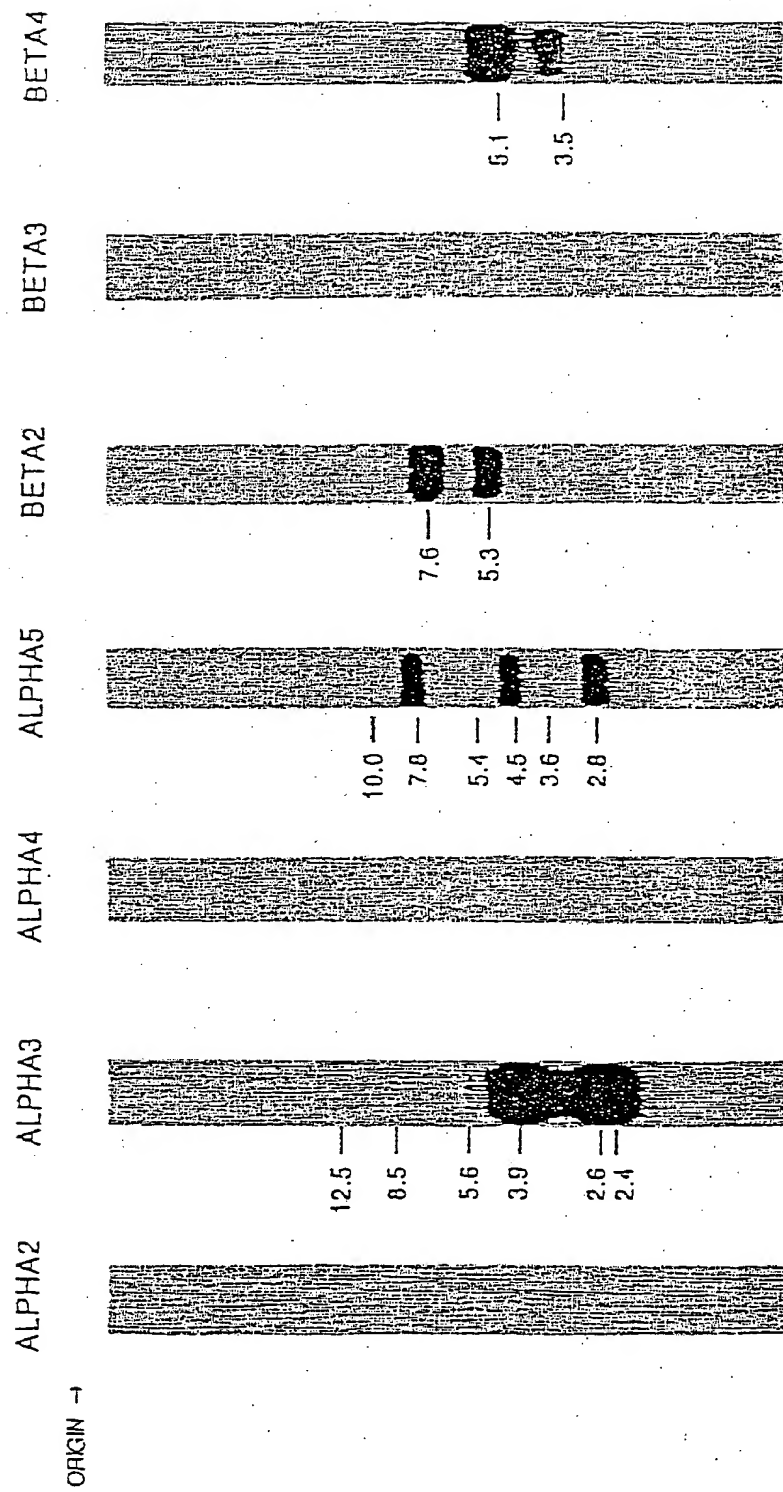


FIG. 31

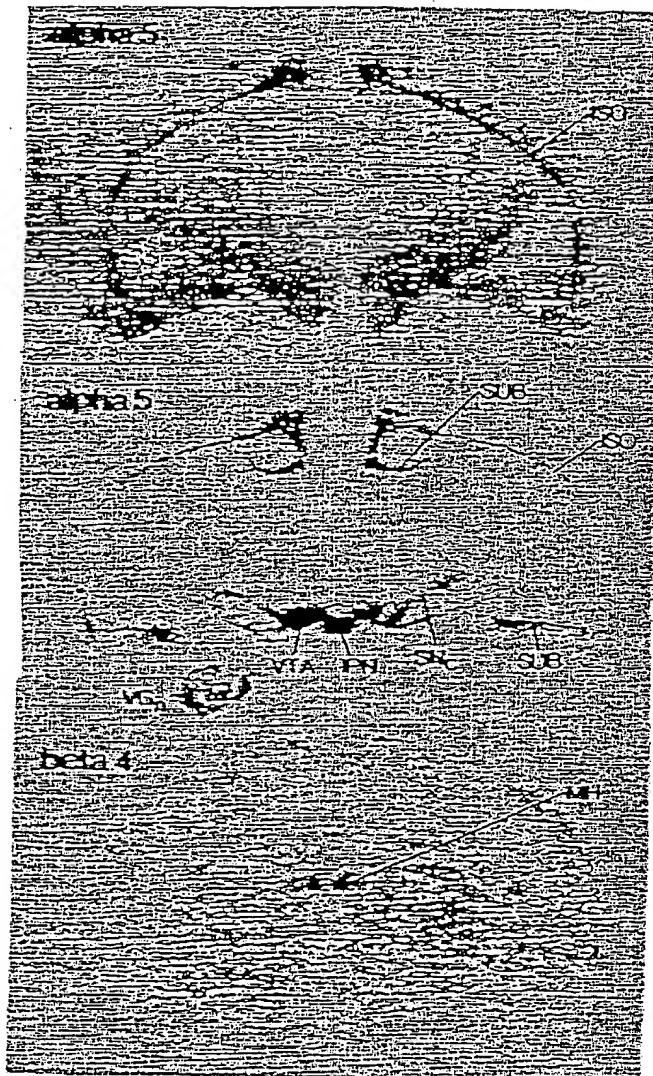


FIG. 32



North2South Direct Stringency Wash

Notes/Conditions	Tips	Formula	References	Links/Products	Contents
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North2South Direct Stringency Wash

Stringency wash and final wash buffers are prepared from 20X SSC and 10% SDS provided in the North2South Direct Kit. Buffers should be prepared fresh using clean labware and quality-grade water (nuclease-free). Using old wash buffers risks bacterial contamination common in dilute buffer systems without preservative especially when incubated at high temperature. Prepare enough stringency wash and final wash buffer solution for 3 washes of 20 ml/100 cm² each. Wash volumes of 40 ml are sufficient when using the 50 ml centrifuge tubes. Stringency may be controlled by buffer salts or temperature. The standard North2South Direct Protocol recommends using a low stringency wash buffer, 2X SSC, 0.1% SDS warmed to the same temperature used for hybridization. High stringency wash buffer, 0.5X SSC, 0.1% SDS, or ultrahigh stringency wash, 0.1X SDS, 0.1% SDS may be used if needed. It is important to verify buffers and incubator temperature with calibrated thermometers. Use of lower temperatures may cause background and nonspecific detection; higher temperatures may wash off target. Low stringency may be more important for smaller probes. High stringency conditions may be required for plaque lifts or crude samples. Be aware the dry and water bath incubators differ in time required to bring buffer to temperature. The stringency wash buffer may require 1-2 hours to come to temperature for larger volumes. If rapid warming is required, the buffer can be warmed in 40 ml aliquots in 50 ml centrifuge tubes. After hybridization, transfer the blot to a clean wash container filled with stringency wash buffer. Wash 3 x 5 minutes with the stringency wash buffer at the same temperature used for hybridization. Do not exceed 15 minutes or reduced signal may occur. Next, wash 3 x 5 minutes with 2X SSC at room temperature. Nonspecific signal is rarely observed. However, stringency wash buffer may be altered if desired.

Notes/Conditions

Nucleic acid thermal hybrid stability is expressed in terms of its melting temperature T_m . Factors that influence the hybridization and washing include temperature, ionic strength, destabilizing agents, mismatched base pairs, duplex length and viscosity and base composition. Stringency washes are controlled by altering temperature, salt concentration and destabilizing agent concentration. High salt concentration favors hybridization reactions. Optimal hybridization is reported at 1.5 M Na⁺ (Labfax). Lowering the salt or increasing detergent concentration may reduce nonspecific hybridization and background. Increasing temperature should also decrease nonspecific hybridization and background. ([back to top](#))

Tips

- Use nuclease-free grade water (filtered or DEPC) for wash buffer preparation.
- Normal wash conditions use 2X SSC, 0.1% SDS (at hybridization temperature) followed by 2X SSC (room temperature). High stringency washes (i.e., 0.1-0.5X SSC, 0.1% SDS) may reduce background for crude samples or plaque lifts. Low stringency (i.e., 5X SSC, 0.1% SDS) may help smaller probes or low T_m probes to remain hybridized.
- Temperature as well as salt concentrations may be adjusted to control stringency washes. Lowering the temperature may favor nonspecific hybridization while raising the temperature may wash hybridized probe from target.
- Do not exceed 3 x 5 minute washes. The longer exposure to heat in the absence of stabilizers can damage the enzyme.
- Make sure the hybridization oven and buffers are at appropriate temperature by measurement with a calibrated thermometer. ([back to top](#))

Formula

20X SSC 0.3 M trisodium citrate, 3 M NaCl, pH 7, 0.2 μ m filtered
20X SSPE provides better buffering for formamide based systems. This system is not useful for North2South Direct due to the fact that HRP is not stable in organics. ([back to top](#))

References

1. Brown, T.A. (1991). Molecular Biology LabFax. Bios Scientific Publishers Limited.
2. Sambrook, J., *et al.* (1989). Molecular Cloning: A Laboratory Manual. 2nd ed, Cold Spring Harbor Laboratory Press.
3. Ausubel, F.M., *et al.* (1995). Current Protocols in Molecular Biology. John Wiley & Sons, Inc. ([back to top](#)).

Links/Products

20X SSC and 10% SDS? Will we carry separately? Other? ([back to top](#))
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Other:

[Endogen](#) [Pierce Nucleic Acid Technology](#) [HyClone](#) [Atos Medical](#)

DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection

last update September 7, 2000



Hybridization stringency

A very important factor for a successful blotting experiment is the stringency of the hybridization conditions. If the stringency is too low, unspecific hybridization may occur. On the other hand, if the stringency is too high only weak or even no signals may be observed.

For any hybridization, stringency can be varied by manipulation of three factors: temperature, salt concentration, and formamide concentration.

Factor	Influence
Temperature	High temperature increases stringency Low temperature decreases stringency
Salt concentration	High salt decreases stringency Low salt increases stringency
Formamide	Decreases melting point of DNA, thus lowering the temperature at which a probe-target hybrid forms. Thus, rising amounts of formamide increase stringency.

Which hybridization conditions are optimal strongly depends on the type and the GC content of the hybrids. RNA:RNA and RNA:DNA hybrids will require higher hybridization temperatures than DNA:DNA hybrids. In general, the relative strength of different hybrids is **RNA:RNA hybrids > RNA:DNA hybrids > DNA:DNA hybrids**. As a rule of thumb, for mammalian targets containing 40% GC, the optimal hybridization temperatures in the presence of DIG Easy Hyb or 50% formamide are:

For This Type of Hybrid	T _{opt} is
DNA:DNA	37 - 42°C
DNA:RNA	50°C
RNA:RNA	68°C

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